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ON THE REGULATION OF RESPIRATION. By HENRY HEAD, M.A. (Pl. I. to IX.).

(*From the Physiological Laboratory of the German University in Prague.*)

PART I. Experimental.

§ 1. *The Method of Registering the Respiratory Movements.*

The many methods now in use for studying the movements of respiration fall naturally into two main groups according to whether the movements of the air to and from the lungs or the contractions of the diaphragm are recorded. Those of the first class trace their origin from the "tambour enregistreur" of Marey. The original method of using this instrument for recording the respiratory movements was somewhat as follows. A T shaped cannula was inserted into the trachea and one of the limbs was connected with a tambour. The other limb was either left entirely open or was closed just sufficiently to allow the tambour to answer to the movements of the expired and inspired air. This method is so inefficient that it was soon modified as follows. A flask of considerable capacity was connected on the one hand with the tracheal cannula and on the other with the tambour. The animal now breathes out of and into the flask and the variations in the volume of the air of the flask are registered by the rise and fall of the lever of the tambour. But the tubing connecting the flask with the tracheal cannula must be extremely short if dyspnoea is to be avoided. I found that three inches of tubing attached to a cannula of at most an inch in length were quite sufficient to cause distinct dyspnoea. In order to obviate this error and to shorten the connexion between the flask and the trachea as much as possible Hering caused a flask to be constructed with the tracheal cannula let directly into one of its sides.

Gad[1] has recently introduced a very accurate method depending on a modification of this principle. By means of his aeroplethysmograph

[1] "Die normale Athmung." *Archiv f. Physiologie* v. E. du Bois Reymond, 1880.

he registers not only the amount of the air passing in and out of the lungs at each breath but also any change in their capacity which may take place in consequence of a tonic contraction of the inspiratory or expiratory muscles.

Knoll has registered the variations in the thoracic pressure by inserting a cannula into the pericardium, and Bernstein[1] has used the oesophagus for the same purpose.

Knoll[2] has also described a very useful piece of apparatus which has been in use in this laboratory for many years. It consists of a glass box in which the whole animal is enclosed. A cannula perforates one wall of this box and is connected with the trachea so that the animal breathes the air outside the box whilst the movements of the air inside the box are registered by means of a tambour.

However, all these methods, admirable as most of them are, failed in my experiments for the following reason. All "pneumatic" methods register simply the increase and decrease in the volume of the lungs, however caused. Now I purposed to continue the experiments of Hering and Breuer[3], who used artificial alterations in the volume of the lungs as a means of stimulating the end organs of the vagi. But it is obvious that if, for instance, the lungs are inflated, the lever of the registering apparatus will be enormously affected and the whole curve will be altered by the very stimulus whose effect on the respiration we wish to study. Hering and Breuer in their experiments registered the respiratory movements with either a mercury or Fick manometer. A T shaped cannula was inserted into the trachea and was connected with a mercury manometer. The third limb was left open to the air. Through this third limb air was forced into the lungs, the mercury rose in the manometer and owing to the standstill in the respiration produced by the inflation remained for some considerable time steady at the same level. The writing point then began to rise slowly, but suddenly sank on the appearance of the first inspiration. The third limb of the T cannula was now opened, for the animal could not breathe in the confined space at its disposal. This experiment was sufficient to show that artificial

[1] "Ueber die Einwirkung d. Kohlensäure d. Blutes auf d. Athemcentrum." *Archiv f. Physiologie* v. E. du Bois Reymond, 1882. Bernstein, however, mentions the fact that Ceradini had already used this method for investigating the changes in thoracic pressure.

[2] Knoll. "Ueber Reflexe a. d. Athmung bei Zufuhr flüchtiger Substanzen." *Sitzb. d. k. Akad. d. Wissensch.* iii. Abth. 1874.

[3] Two papers on "Die Selbststeuerung d. Athmung durch den Nervus Vagus." LVII. Bd. *Sitzb. d. k. Akad. d. Wissensch.*, 1868. April and November.

inflation of the lungs caused a standstill of the respiratory movements, and it is by no means difficult by watching the movements of the diaphragm to assure oneself that collapse of the lungs causes an inspiratory movement. But such a method although amply demonstrating the result of a single inflation is quite inadequate for the investigation of the complicated phenomena following the periodic repetition of such stimuli.

Some method depending on the registration of the movements of the diaphragm seemed to offer the best chance of success for work on these lines. But all the phrenographs in use labour under the same disadvantage, that any movement of the animal, whether passive or active, tends to cause such alterations in the curve that it is impossible to compare the curves obtained before and after such a disturbance. Suppose for instance that the lever of a Rosenthal phrenograph is inserted between the liver and the diaphragm and the point is marking regularly on the drum; on inflating the lungs the diaphragm and with it the lever is passively pushed downwards towards the abdomen; the pause caused by the inflation gives place to an active expiration and the lever is again passively pushed from its previous position—this time in an upward direction. These passive movements so obscured the phenomena following artificial variations in the volume of the lungs that a new method had to be found for registering the respiratory movements.

When the diaphragm of a rabbit is examined from the abdominal side the strong development of the two anterior limbs is very noticeable. They extend along the posterior surface of the xyphoid process and are comparatively sharply separated from the rest of the diaphragm. On dividing the skin and opening the abdomen in the middle line the xyphoid process comes into view, covered on each side by the lower part of the origin of the pectoralis major. On separating this part of the muscle by passing the scissors upwards on each side of the bony portion of the process the two slips of the diaphragm are seen each covered by a small band of muscle. This must be carefully removed and the two anterior limbs of the diaphragm are then laid bare, covered in the middle line by the bony portion of the xyphoid process. Each limb is supplied with blood from above by a small branch of the musculo-phrenic division of the internal mammary artery. This branch together with its accompanying vein runs alongside the bony part of the process, sending off a branch to supply the muscle of its side about midway between the ensiform cartilage and the base of the xyphoid process. By carefully inserting a fine pair of scissors between the muscles and the bone beginning in the

neighbourhood of the cartilage it is possible to separate the two limbs of the diaphragm without much injury to their tissue and without dividing the vessels which supply them. After the bone is thus prepared it is separated from the ensiform cartilage to which the muscles remain attached. The muscles are then separated as carefully as possible from the under surface of the bony process. We now have a muscular band slightly attached at the one end to the anterior wall of the thorax and in connection with the ensiform cartilage at the other end. The injury suffered by the muscles is confined to the small portion in the middle line where the two slips join one another and send a few fibres to be attached to the bony part of the xyphoid process. Neither the nerves nor the blood vessels supplying these muscles are affected if the operation has been properly carried out. But these limbs of the diaphragm no longer have a fixed origin for they arise from the ensiform cartilage. The ensiform cartilage was the fixed point from which by their contraction they pulled the diaphragm forwards and downwards. It thus becomes necessary to give them an artificial fixed point if we are to register their movements with accuracy. If they are fixed to the chest wall every inspiration will cause the cartilage to approach the sternum, owing to the contraction of the muscles, and the contraction curves will no longer be disturbed by the contraction of the remaining portions of the diaphragm. The muscles are best fixed to the chest walls as follows. A thread, carrying at the one end a small flat button, is passed from the abdominal side through the diaphragm and the walls of the thorax between the fifth and sixth ribs. As soon as the thread is drawn tight the button is pressed firmly against the muscle and thus fixes it to the anterior wall of the thorax. A similar operation is performed on the other side and the two threads are knotted together over the front of the sternum.

A thread is now attached to the ensiform cartilage and after passing over a pulley is attached to an ordinary writing lever such as is used to register the contractions of a frog's muscle. This marks on the smoked surface of the drum of a Hering kymograph.

Now as the fixed point of this system lies on the wall of the thorax anything that causes an alteration in the position of the chest wall will also affect the lever in connection with the muscle. It is therefore important to know how far the excursions of the lever are due to passive alterations in the position of the fixed point and how far they are due to active contractions of the muscles. An exactly similar lever is therefore attached to the knot formed by the union of the two threads and

registers all movements of the chest wall whether passive or active. Now, when the chest wall moves, both levers will describe a similar curve, whereas a contraction of the diaphragm will only appear on the curve drawn by the lever in connection with the two separated slips of muscle. The action of, and necessity for, such a control is especially evident in the experiments on the effect of dividing the vagi.

To pass on to the disadvantages of this method, I must first of all mention that the muscles can never be prepared without some injury to their tissue. But the injury should only affect the inner (median) edge of each strip, and the vitality of the muscle seems but little affected by so small an injury. The real disadvantage caused by this injury lies in the formation of small blood-clots in the injured portion, which tend to obscure any contraction which lasts for an unusually long time. It is always advisable to gently touch the muscles with a small piece of sponge before beginning any series of experiments, and it is always well to so weight the lever that the muscles return to the same point after each normal inspiration. It is also a disadvantage that the curve is affected by the movements of the thorax, but in the rabbit such movements so rarely occur that they are of but little moment.

In spite of its apparent simplicity the preparation of the muscles at first presented considerable difficulty and it was some time before they could be prepared in a condition of unimpaired vitality. But even after considerable practice cases now and then occur, though rarely, where owing to degeneration in the muscles themselves or to some peculiarity in their nerve or blood supply the preparation entirely fails.

In every case the animals used were rabbits, and in every case they were more or less deeply narcotized. At first I injected chloral through a cannula tied into the internal jugular vein. A peculiar form of restlessness, which mostly took the form of periodic movements of swallowing, made its appearance so often that I gave up this method. In such cases I noticed that the urine became tinged with blood and an investigation of the kidney revealed clots of blood between the pyramids of startling size. I have since injected chloral under the skin and, since resorting to such hypodermic injections, this restlessness has entirely ceased to appear. The amount injected varied according to the experiment to be performed. Gad recommends 2 c.c. of a 1:2 solution per kilo. body weight. This produced a very deep narcosis, and is in my opinion too strong a dose for many experiments.

§ 2. *Normal Respiration.*

On examining the normal respiratory curve traced by the aeroplethysmograph or any other instrument which registers the movements of air to and from the lungs the following points will be noticed. The inspiration is sudden and sharp and its tracing is but slightly curved; the expiratory curve is at first very quick and sharp but rapidly becomes slower, so that the last part of the tracing is strongly curved; the expiration may then end by tracing a horizontal line.

However, this curve represents the movements of the air during respiration and not the actual movements of the respiratory muscles. We should therefore expect that such curves would differ slightly from those obtained by registering the actual movements of a portion of the inspiratory musculature. Curve x (Plate I) has been chosen as more or less typical of the normal movements of the muscular slips, used in my method, during the various phases of respiration. As the lever by which this curve was traced described an arc when the drum was at rest I have drawn lines at various points on the curve to show the curves which would have been produced by the lever had the drum not been in motion.

It will be seen that the contraction begins rapidly but soon becomes slower. The lever is still rising with considerable rapidity when the contraction suddenly ceases. The first portion of the descending curve is so rapid that it almost coincides with the line traced by the lever when the drum is at rest. The muscles continue to elongate but with decreased rapidity until they are completely relaxed. However it must not be forgotten when examining the fall of the lever that the downward curve will always appear steeper than is warranted by the actual rate of elongation in the muscles, in consequence of the weight attached to the lever. The writing point now traces a horizontal line representing the complete elongation of the muscular tissue.

I have already used the words inspiration and expiration in what may be considered a somewhat loose manner, and shall proceed to define what is understood in this paper by these terms. An inspiration is taken to mean a rhythmic contraction of the inspiratory muscles from its beginning to the moment when the muscles begin to relax again. An expiration on the other hand begins when the muscles begin to relax and ends with the birth of the next inspiration. Such an expiratory period may consist of three stages. Firstly, the time taken up in the relaxation of the muscles after the inspiratory contraction. Secondly,

the pause—the stage during which the muscles remain completely relaxed. Lastly, under certain circumstances this pause may pass gradually into an active expiration, caused by the contraction of the abdominal muscles. I have refrained from dividing the respiratory period into inspiration, pause and expiration, for I think a closer analysis will show that, at any rate in the rabbit, the active expiration is but a stronger form of the same phenomenon which shows itself normally as a pause. The inspiration bursts through the pause and the inspiratory muscles contract with great rapidity at the beginning of the inspiratory phase. On the other hand if an active expiration appears it does not arise suddenly like the inspiratory contraction but the pause appears to give place to a slow but ever increasing contraction of the abdominal muscles. From the summit of this contraction arises the next inspiration by a sudden contraction of the inspiratory muscles following immediately on a sudden elongation of those of the abdomen. From the moment that the inspiratory curve ceases to rise to the birth of the next inspiration there is no violent change such as is seen at the close of the active expiration and beginning of the next inspiration.

I have just pointed out that of the three stages into which the expiratory period may be divided the last—active expiration—is often absent in the rabbit. Now we also find that under certain conditions the second stage—that occupied by the pause—also fails to make its appearance. The muscles begin to relax after the inspiratory contraction, but before they have elongated completely the next contraction begins (cf. Curve i, Plate I). Under such circumstances the muscles are never completely elongated, even at the most extreme point of the expiratory period, and remain in a permanent condition of tonic contraction. On this tonic contraction the rhythmic movements are superposed. Gad[1] has already shown that under certain conditions the volume of the thorax is permanently enlarged by the tonic contraction of the muscles of the chest wall, and this tonic contraction of the diaphragm is the expression of the same phenomenon in the greatest of the inspiratory muscles.

It might be objected that the form assumed by the curves of elongation and contraction were not the expression of the activity of the respiratory centre but were in part due to some peculiarity in the activity of the two muscular slips. In order to examine this possible source of error I prepared and stimulated the phrenic nerve on one or on both sides with an interrupted current. If the drum was revolving

[1] "Die Regulirung der normalen Athmung," du Bois Reymond's *Archiv* 1880.

at the ordinary pace the rise and fall of the lever, at the beginning and end of the stimulation, were so sudden as to be indistinguishable from an instantaneous rise and fall (Curve vii, Plate I). It was only when the drum was revolving at a pace enormously in excess of any used in my experiments that the line of the contraction curve appeared anything but instantaneous. The rapidity and promptness with which the muscles contract is beautifully shown by allowing the divided phrenic of the left side to lie on the heart, when every heart beat is followed by a contraction of the diaphragmatic muscles of the left side. On Curve vii, Plate I. the left phrenic nerve was stimulated electrically during the normal inspiratory contraction, and the difference in rate between the rise of the curve under the nervous influence of the inspiration and in consequence of the artificial stimulation is very distinctly shown. The second portion of this curve shows the result of stimulating the phrenic nerve during the expiratory pause. Again, in order to make sure that the contraction remainder which appeared on certain curves was really due to a tonic influence from the respiratory centre I divided the phrenics and the levers at once full to a position representing the complete elongation of the muscular slip.

§ 3. *Division of the Vagi.*

If we turn to the literature on the Physiology of Respiration we find that, next to artificial stimulation of the vagi, division of these nerves plays the largest part in the experiments of most observers. Yet there was scarcely any experiment which gave such contradictory results. If the vagi are cut or ligatured in the ordinary way it is quite impossible to prophesy the results. Sometimes the breathing becomes irregular and the animal restless, sometimes division is followed by long expiratory pauses, and sometimes the breathing at once assumed the type which in all cases ultimately followed division of the vagi. But when the vagi are divided in the ordinary way the experiment is complicated by the stimulation of the central end of the cut nerves; not only do we remove any influence which the vagi may normally exert on the breathing but we stimulate them at the same time. However Gad[1] has shown that we possess a method of dividing the vagi without stimulation. He states that if they are frozen all impulses from the periphery are at once cut off without the slightest stimulation to the nerves themselves and, as

[1] "Die Regulirung d. normalen Athmung."

far as my experience goes, I can entirely confirm his statement. For if one vagus is frozen a stimulus applied on the peripheral side of the frozen point is totally without effect on the breathing. If the frozen nerve is removed from the freezing apparatus it can be frozen again on the central side of the frozen point without causing any alteration in the respirations, although a mechanical or electrical stimulus is as active as ever.

But it would seem that the vagi are comparatively insensible to mechanical stimulation, and I think we must seek the cause of the extreme variability in the phenomena, which follow their division in the ordinary way, elsewhere than in the assumption of mechanical stimulation. Knoll[1] has shown that it is extremely easy to stimulate the vagi by closing the current in the divided nerve itself. If the nerve be cut and allowed to fall into the wound in the neck or if it be moistened with "physiological" salt solution the nerve current is closed and all the phenomena of a slight expiratory stimulation make their appearance. If a small piece of dry gutta-percha or other non-conducting substance be laid under the prepared vagus, division whether by freezing or with a sharp pair of scissors always causes the same result. But if the gutta-percha is now moistened with salt solution or if the cut nerve is allowed to fall back into the moist wound the scene at once changes. Expiratory pauses, with or without irregularity in the breathing, at once make their appearance. Exactly the same changes appear when the nerve is taken from the freezing apparatus and allowed to fall back into the wound. In fact electrical stimulation of the divided nerves by their own current seems to be the most important factor in the production of the phenomena which are said by most observers to follow the division of these nerves. Now although it is possible to divide the vagi with a sharp pair of scissors without stimulating them, the precautions necessary to avoid stimulation are so many that I far prefer Gad's method.

The apparatus I have used for cooling the vagi below 0° C. differs slightly from that used by Gad. An ordinary glass filter of suitable size is divided just above the tube and the bottom closed with a flattened cone of copper into which two bent copper wires are soldered. The filter is filled with pounded ice and salt and in a few seconds the temperature of the wires has sunk below 0° C.

Another method for dividing the vagi which is sometimes of great

[1] "Beiträge zur Lehre v. d. Athmungsinnervation" Mitth. I. *Sitzb. d. k. Akad. d. Wissensch.* Bd. LXXXV. Abth. iii.

value is as follows. It is well known that a nerve can be completely anaesthetized by subjecting it to the vapour of ether[1]. The vagus is placed in a tube of indiarubber so constructed that ether vapour can be passed over it without affecting the atmosphere which the animal is breathing. In a few seconds the nerve is completely anaesthetized and is incapable of conducting impulses from the periphery. Now by blowing air through the tube, instead of ether vapour, the nerve completely recovers its conducting power and seems to be totally unaffected, even if the process be repeated several times, provided no fluid ether has entered the tube.

The results which follow division of one vagus are generally very transitory. As soon as the nerve is completely frozen the inspirations become stronger. The rate of contraction is the same, but the strength and duration of the inspirations are increased and the curves become somewhat rounded at the summit (Curve i, Plate I). The expirations may be somewhat longer but generally remain unaltered. The animal will continue to breathe in this manner for a considerable time if left undisturbed. But after the space of about half an hour or even less, the breathing has almost completely returned to the original form although the vagus is completely divided. However division of one vagus is not infrequently followed, not only by an increase in the strength of the rhythmic inspiratory contractions, but also by a shortening of the expiratory period and the appearance of a varying amount of tonic contraction. But if only one vagus has been divided this tonic contraction rapidly wears off and ultimately, if the animal be left long enough, the breathing almost completely returns to the form it assumed in the intact animal. Thus division of vagus may cause changes of two distinct kinds. The inspirations are invariably more or less increased, both in strength and duration, but the expirations may either remain unaltered or, at any rate in the first few moments, be considerably shortened.

Now if the second vagus be frozen we see exactly the same changes as followed division of one vagus although in a more pronounced form (Curve ii, Plate I). As soon as the second nerve is divided the muscles contract to an extent far exceeding that of any normal inspiration. The inspiration forms a broad flat crested curve closely resembling those seen on closing the trachea of the same animal during the expiratory phase.

[1] Cf. the recent paper by Biedermann. *Sitzb. d. k. Akad. d. Wissenschaft.* Bd. XCVII. Abth. iii. März 1888.

A sudden fall proclaims the advent of the first expiration. Here again we find that removal of the influence which the vagi normally exert on the breathing leads to an increase in the force and duration of the inspiratory contractions but leaves the rate of contraction unaltered. The muscles may elongate completely after each contraction, in which case the expiratory periods are generally a little longer than normally. But far more often the elongations are incomplete and the base line of the curve appears to be raised. Now Gad has shown that in the rabbit the thorax generally assumes a permanently inspiratory position after division of the vagi. It therefore seemed possible that this apparent rise of the base line of the curve might be purely passive and be caused by the position of the thorax. I therefore divided the vagi, and as usual the base line of the curves traced by both levers rose, though the rise of the lever in connection with the muscular slips was distinctly more pronounced. Both phrenic nerves were then cut as rapidly as possible and the muscles at once elongated, showing that they were previously in a condition of permanent tonic contraction (Plate I, Curves iii and iv).

After the removal of the vagi it is no longer possible for a change in the volume of the lungs to affect the respiratory centre. We might therefore suppose that the form which the breathing assumed after division of these nerves was the expression of the independent activity of the respiratory centre. But Markwald[1] has pointed out that there is still another factor to be taken into account. If the brain be separated from the medulla just above the auditory striae a rabbit will continue breathing rhythmically for a very considerable time. The changes produced by this operation are very different in different cases. The breathing either becomes rapid and the inspiratory contractions are increased in strength or it may become laboured, both expiration and inspiration being increased in strength and duration. If the cut has passed a little too low the animal lies perfectly passive, and although it does not breathe spontaneously it is the most perfect reflex machine. The slightest diminution in the volume of the lungs evokes a well formed inspiration. However in every case division of the vagi causes the same marked change. Directly the second vagus is divided the muscles begin to contract and an inspiration of enormous duration is produced (Curve v, Plate I). In a normal animal an in-

[1] "Die Athembewegungen und deren Innervation beim Kaninchen." *Zeitschr. f. Biologie* Bd. v. (N. F.) 1886.

spiratory contraction after division of the vagi lasts at most five seconds—and even this is very long. If the brain has been previously separated from the medulla the inspiration which follows division of the second vagus frequently lasts 40 sec. and according to Markwald may reach the enormous duration of one minute seventy-five seconds. But here again a close examination of the curve reveals the fact that the rate of contraction of the inspiratory muscles remains unaltered although the strength and duration of the contraction are enormously increased. This strong inspiration is put an end to by a quick elongation of the muscle. Both thorax and diaphragm ultimately return to a condition of complete rest and the abdominal muscles then begin to contract slowly. When this active expiration has reached a considerable height the abdominal muscles suddenly relax and the inspiratory muscles at once contract violently, producing a second strong and long inspiration. It is a noticeable fact that, when the brain has been separated from the medulla previously to the division of the vagi, each expiration produces a complete return to the state of rest of both thorax and diaphragm. I have never seen even traces of a tonic contraction of the inspiratory musculature.

The first inspiration after dividing both vagi deserves closer attention, especially as Gad scarcely notices it in his description. It mostly far exceeds in duration though not in height any inspiration which follows it. The expiratory elongation which follows it is mostly very incomplete, and if a tonic contraction appears after dividing the vagi it is always most marked between the first and second inspirations. The duration of this first inspiration is intimately connected with the appearance of an inspiratory tone. The same influences which affect the one also affect the other. If the animal is narcotized with morphia or is very deeply under the influence of chloral or if it has been in any way maltreated, we invariably find that the tonic contraction of the inspiratory muscles is absent and that the first inspiration which follows the division of the vagi differs little from those which come after it. If the vagi are divided whilst the animal is breathing an atmosphere of hydrogen or is in any way dyspnoeic both phenomena are again absent. It is therefore no wonder that a tonic contraction is absent when the vagi are divided after the separation of the brain from the medulla, for after the enormous inspiration which follows this operation the centre must be in a condition of violent dyspnoea. In fact we find that under such conditions if the animal be left to itself it gradually dies of slow asphyxia. On the other hand the most favourable conditions for the

appearance of an inspiratory tone and a long primary inspiration are a light narcosis and the least possible exhaustion. The fact that the animal is breathing an atmosphere of pure oxygen when the vagi are divided is not of the slightest influence on either the tone or the duration of the first inspiration except in as far as it assures the absence of dyspnoea. The remaining factors which tend to prolong the inspiration which immediately follows division of both vagi I shall discuss in a further section.

A peculiar phenomenon must now be described which sometimes makes its appearance after the vagi have been frozen. If the vagi are removed from the freezing apparatus ("Thermode"[1]) as soon as they are frozen, by means of a paint-brush moistened in salt solution, they will regain their conducting power after a time although they have been completely frozen. If the nerves are allowed to lie in the wound so that they become completely thawed and are tested at stated intervals (say of a minute) by inflating the lungs, a stage appears in which the inflation instead of inhibiting the inspirations actually shortens the expiratory period (Curve vi, Plate I). The strength of the inspiratory contractions is not increased but their duration is prolonged, and owing to the incomplete expiratory elongation a tonic inspiratory contraction appears during the inflation. After about an hour and a half this phenomenon gradually disappears and inflation of the lungs produces the normal effect. Thus if the vagi are carefully removed from the freezing apparatus as soon as they are frozen three stages appear. Firstly, a considerable time (10 min.) during which inflation of the lungs produces no effect at all—the vagi have lost their conducting power. Secondly, a stage during which an inflation produces an inspiratory effect; and thirdly, one in which inflation of the lungs produces the usual inhibition of inspiration. If however the vagi are allowed to remain some time (10 sec.) on the thermodes this paradoxical effect ceases to make its appearance when the nerves are removed from the freezing apparatus. It entirely fails to appear during the recovery of the vagi after they have been anaesthetized with ether vapour, and I am totally at a loss to account for its appearance. That this phenomenon is due to the vagi and not to some other nerve is proved by its immediate disappearance when the vagi are divided with the scissors. That it is not due to the alteration in blood-pressure caused by the

[1] Gad calls the two bent wires on which the nerves are frozen "Thermodes." "Die Regulirung der normalen Athmung."

inflation is shown by its ceasing to appear after the vagi have been cut through below the frozen spot although inflation of the lungs is still followed by the same change in the blood-pressure.

To sum up the results of the experiments described in this section:

(1) We must be careful in dividing the vagi to guard against the stimulating effect which follows closure of the nerve current in the central end of the cut nerve.

(2) Freezing the vagi does not stimulate them, and the mechanism by which the freezing is effected has the additional advantage of preventing the closure of the nerve current.

(3) Division of the vagi without stimulation invariably produces an overpowering inspiratory effect on the breathing. The vagi normally exert an influence on the breathing, the absence of which shows itself in increased inspiratory activity of the respiratory centre, both tonic and rhythmic. There is no increase in the rapidity with which the inspiratory muscles contract but only an increase in the strength and duration of the contraction.

(4) The tonic inspiratory contraction wears off after a time and we notice that the blood in the left side of the heart is distinctly darker than normal. This seems to show that the respiratory movements which follow division of the vagi are unable to keep the blood up to the normal standard of aeration. The appearance of an inspiratory tone is very dependent on the condition of the centre. Deep narcosis, dyspnoea or exhaustion of any kind tend to prevent its appearance.

§ 4. *The Effect produced by increasing the Duration of the Normal Action of the Vagi.*

We have just seen that the vagi undoubtedly exert some influence on the respiratory centre even during normal respiration. Let us assume with Hering and Breuer and with Gad that this influence is due to the effect produced on the end organs of these nerves by the periodic alterations in volume which occur during normal breathing. Now we obviously have the power of artificially altering this normal influence in two directions. We can either allow the lungs to expand or collapse under the influence of the normal respiratory movements and then by closing the trachea keep them for a greater or less time in one or other condition. By this means we increase the duration of the influence normally exerted by the vagi during respiration. Or,

secondly, we can actually inflate the lungs above the normal volume and allow them to collapse completely by puncturing the thorax. Here we increase the strength of the influence normally excited by the vagi.

If the trachea is closed at the moment that a normal inspiration has reached its height the breathing remains for a short time unchanged (Curve ix, Plate I). The inspiratory contraction ceases and the muscles elongate in the same way and at the same rate as normally. Then follows the usual pause, and it is here that we first notice anything unusual. The pause is enormously prolonged and, in those cases where a tonic contraction was present during the expiratory period, the muscles continue to relax and finally reach a condition of complete elongation. After a varying interval the pause is broken by a strong inspiratory contraction, considerably stronger than the normal inspirations. The elongation which follows is again complete, and a second long pause follows, which is however shorter than the one which preceded it though longer than the normal pauses. If the tracheal cannula is now opened the respiratory movements become very rapid. The expiratory elongations are frequently incomplete and an inspiratory tone appears during the first few moments following the opening of the trachea.

If on the other hand the trachea is closed during the expiratory pause the breathing suffers the converse change (Curve viii, Plate I). The pause during which the trachea is closed lasts the usual time and is followed by an inspiratory contraction of the normal rapidity. But when the inspiration has reached the normal strength, instead of ceasing abruptly, it goes on increasing until the curve reaches a point far above the crests of the normal inspiratory contractions. As the contraction decreases in rapidity, the curve gradually bends over until the lever traces an almost horizontal line. Thus closure of the trachea in the expiratory phase of respiration causes an increase in the strength and duration of the inspiratory contractions. This strong inspiratory contraction is followed by a quick and complete elongation, which is succeeded by a pause equal to, or at any rate not more than twice, the duration of the normal pause. From this pause arises a second long and strong inspiration closely resembling its predecessor. If the trachea is now opened the breathing resumes the normal type and we never find even a trace of the peculiar shortening of the expiratory period which is so common when the trachea is opened after closure in the inspiratory phase of respiration.

These experiments show that, as Hering and Breuer pointed out,

the stimulus to the vagi is not simply caused by the expansion of the lungs but by their expanded condition. For in every case the expansion was that caused by a normal inspiration and yet the breathing was affected as long as the lungs were kept in the expanded position.

Though the effect produced by closing the trachea during the different phases of respiration depends on the condition of the lungs and not on the act of collapse or expansion, yet the breathing does not cease to be rhythmic. Closure in the inspiratory phase produces a long pause, but this pause is always ultimately broken by a strong inspiration. Similarly the strong inspiration which follows closure in the expiratory phase is always followed by an expiration. Thus although the lungs remain permanently in a condition of collapse or dilatation the centre still continues to send out rhythmical impulses although these impulses are much modified by the effect produced by the condition of the lungs.

To sum up the results of the experiments described in this section.

(1) Closure of the trachea in the inspiratory phase of respiration causes a decrease in the inspiratory activity of the centre, whilst closure in the expiratory phase causes an increase both in the strength and duration of the inspiratory contractions.

(2) This effect is produced by the volume assumed by the lungs and not by the act of expansion or collapse.

(3) In spite of the fact that the lungs remain permanently dilated when the trachea is closed at the height of the inspiratory phase, the pause so produced is broken by an inspiration. Conversely in spite of the permanently diminished volume which the lungs assume when the trachea is closed in the expiratory phase the strong expiration so produced is cut short by an expiration. Thus although the lungs do not materially alter in volume the activity of the centre, though profoundly altered, still remains rhythmic.

§ 5. *Increase and Diminution in the Volume of the Lungs.*

A. The immediate effect of increasing and diminishing the volume of the lungs.

In the previous section we examined the effect of increasing the duration of the natural stimulus. We now pass on to the results which follow an increase in its strength.

The first result of a gentle inflation of the lungs is an immediate cessation of all inspiratory activity. If the inflation falls in the period represented on the curve by the pause this same pause is immensely prolonged (Curve xiii, Plate I). It can last 15—20 sec. or even longer, but is always sooner or later broken by an inspiratory contraction. If on the contrary the inflation takes place in the middle of an inspiratory contraction this contraction is at once inhibited and the prolonged pause begins. I have already mentioned that under certain circumstances the expiratory elongations are incomplete. If the lungs are inflated under such conditions, the rhythmic inspiratory contractions cease at once and the lever sinks to the point it occupied between any two previous inspirations (Curve xi, Plate I). But if the lungs are allowed to remain in a dilated condition the lever continues to sink steadily, so that if the pause last long enough the muscles finally elongate completely. Thus an inflation of the lungs inhibits, firstly, the rhythmic inspiratory contractions and, secondly, any tonic contraction of the inspiratory muscles that may be present.

I must here reiterate the warning of Hering and Breuer[1] against violent inflation. The inflation must always be made with care; for if the air is violently blown into the lungs an inspiration always precedes the inhibitory pause. This is especially the case with animals that are but lightly narcotized. It is in all probability due to stimulation of some sensory nerve in the body-wall, for I have seen it in but lightly narcotized animals even after division of the vagi. Just such an inspiration appears when any sensory nerve is stimulated electrically[2]. But this is not the only evil effect resulting from violent inflation. Violent distension of the lungs easily leads to lesions in their structure which appear in a post-mortem examination as permanent emphysematous distension of the alveoli.

If the lungs are kept in a dilated condition sufficiently long the pause is ultimately broken by an inspiration. This "interrupting" inspiration appears on the curve as a quick contraction often double the strength of that of a normal inspiration. The contraction is quick but of short duration compared with its strength, and it therefore traces a curve with a very sharp apex. It closely resembles the inspirations which often appear when the trachea is closed in the inspiratory phase and

[1] Hering. *Sitzb. d. k. Akad. d. Wissenschaft.* 1868, Bd. LVII. page 917.

[2] Knoll. "Beiträge zur Lehre v. d. Athmungsinnervation," Fünfte Mittheilung. *Sitzb. d. k. Akad. d. Wissensch.* III. Abth. 1885.

is very different from the strong flat-topped inspirations which follow division of the vagi or closure of the trachea in expiration.

There are two methods by which the volume of the lungs may be diminished. Either the thorax may be perforated and the lungs allowed to collapse completely[1] or the volume of the lungs may be diminished by applying suction to the opening of the tracheal cannula.

Hering and Breuer[2] describe the results which are produced by complete collapse of the lungs as follows:—"Beim plötzlichen Lungencollaps durch Pneumothorax tritt ein mächtiger Inspirationstetanus ein, der, bei Kaninchen bis zu 8 und 10 Secunden Dauer hat und auch im weiteren Verlaufe nur durch kleine Oscillationen des Zwergfells um seine Inspirationsstellung unterbrochen wird, bis allmählig bei wachsender Dyspnoe grössere expiratorische Erschlaffungen des Diaphragmas eintreten."

Although Hering and Breuer did not use a graphic method for recording these results the words I have just quoted are an exact description of the curves I have obtained on allowing both lungs to collapse suddenly. If but one side of the thorax is opened we obtain a similar curve except that the tetanus is very much shorter than when both sides of the thorax are opened (Curve xi, Plate II). But on the other hand the animal recovers quite easily after opening one side of the thorax and there is no necessity to have recourse to artificial respiration, an advantage which will be appreciated later. If but one lung is allowed to collapse the inspiratory tetanus which is produced expands the lung on the sound side of the thorax to a very considerable extent. This expansion undoubtedly produces a depressing effect on the inspiratory activity of the centre and probably accounts for the comparative shortness of the tetanus produced by the collapse of only one lung. I hit upon the following method for obviating the inhibition from the sound lung and at the same time keeping the animal alive without artificial respiration. The right vagus was divided and as soon as the breathing had almost completely returned to the normal type the left side of the thorax was opened. Now in many cases the vagus of the left side but slightly supplies the right lung. Thus under favourable conditions we have almost completely got rid of all possibility of inhibition from expansion of the right lung (Curve viii, Plate II). On perforating the

[1] I need scarcely say that the lungs do not become atelectatic. As soon as the thorax is opened the lungs recede from the body-wall and assume a volume considerably below that which they reach in even extreme expiration. They however still contain air.

[2] Page 5, Bd. LVII. 1868.

left side of the thorax after dividing the right vagus the muscles contracted quickly and remained for over 20 sec. in a condition of tetanic contraction. The first incomplete expiratory elongations then appeared, but it was many minutes before the respiratory movements in any way approached the normal type. Finally the breathing assumed a form differing from the normal in the strength of the inspirations and the presence of a certain amount of inspiratory tone. I may add that the tetanus following collapse of one lung under such conditions is seldom so long as on Curve viii, Plate II which has been chosen for its extremely marked characteristics. Obviously it is now in the power of the experimenter by inflating the lungs of such an animal and then allowing them to collapse to repeat to a certain extent the phenomena attending the original collapse (Curve v, Plate II). But on attempting to reproduce the phenomena of the original collapse by this means, we find that the result of collapse is not always the same. Normally the inflation causes a pause in the breathing which as soon as the lungs are allowed to collapse gives way to a strong tonic contraction; the rhythmic breathing is completely abolished for several seconds. But if the animal is made dyspnoeic this tonic contraction ceases to appear on collapse of the lungs. The more dyspnoeic the animal, the less is the tone which follows collapse, and sometimes the only effect of the return of the lungs after the inflation is to slightly increase the strength of the rhythmic inspiratory contractions. The appearance of the tonic contractions is intimately dependent on the due aeration of the respiratory centre.

I pointed out above that after collapse of one lung the breathing was characterized by a certain amount of tonic contraction of the inspiratory muscles. After a considerable time this tone wears off and the muscles elongate completely after each inspiration. If the lungs are now inflated and allowed to collapse, not only do we observe the phenomena that I have just described but we also find that the tonic contraction has returned. After the immediate effect of the collapse has passed, the muscles still fail to elongate completely after each inspiration.

So far we have considered the effect of what may be called *permanent* inflation and collapse of the lungs. It is of especial importance for the study of the phenomena produced by artificial respiration to determine the effect of what may, in comparison, be called *momentary* expansion and collapse.

A momentary inflation must not be caused by a violent burst of air but the lungs must be gently inflated and allowed to return at once to

their normal volume. If such an inflation falls in the inspiratory period the sudden shortening of the inspiratory contraction is often the only result produced (Curve i, Plate II). If on the other hand the lungs are inflated during the expiratory pause, that pause is mostly considerably lengthened (Curve x, Plate II). If an inspiratory tone is present, a momentary inflation applied during the expiratory phase causes a slight elongation of the muscles, whereas if it falls in the inspiratory period it simply shortens the rhythmic inspiratory contraction and leaves the tone unaffected.

A momentary diminution in the volume of the lungs can be produced by sucking a small quantity of air from the trachea and allowing the lungs to return at once to their normal volume. If such a momentary collapse falls in the period occupied by the pause, an inspiratory contraction at once appears resembling a normal inspiration enlarged in every direction (Curve ix, Plate II). If the suction is applied when a normal inspiration has just reached its height the already rounded curve gives place to a sudden further contraction of the inspiratory muscles, which in its turn describes a rounded curve and suddenly ceases. Thus a second inspiration is produced taking its rise from the crest of the normal contraction of the inspiratory muscles. If the suction is applied during the expiratory elongation before the muscles have completely elongated, a fresh inspiration is produced rising from the still contracted diaphragm.

B. The positive afteraction caused by increase and diminution in the volume of the lungs.

The effect of a momentary increase or diminution is not confined to the period during which the inflation or suction lasted. Even after the lungs have returned to their normal volume the breathing is considerably modified. If the lungs are momentarily inflated during the expiratory pause, not only is that pause prolonged but the next inspiratory contraction is also considerably smaller than usual. Each succeeding inspiration gains in strength, but it is not until the sixth inspiration after an inflation lasting at most 1½ sec., that the inspiratory contractions reach the normal strength (Curve ii, Plate II). This partial suppression of the contractions is frequently accompanied by a lengthening of the expiratory pauses although this is not always the case. On Curve i, Plate II the only effect produced by a momentary inflation was a diminution in the strength of the inspirations.

Now it appeared possible that this diminution in inspiratory activity which followed a momentary inflation might be due to some amelioration in the condition of the air in the lungs. I therefore substituted hydrogen for air as the means of inflation, but exactly the same afteraction appeared after inflation with either gas. In fact it would be impossible to tell on most of the curves which gas had been used in the inflation. However a slight difference sometimes appears in the later stages of the afteraction. For the afteraction, if strong, renders the inspirations so ineffectual that the hydrogen in the lungs is not sufficiently replaced by air and a certain amount of dyspnoea results. Even when air is used some dyspnoea not infrequently appears owing to the smallness of the inspirations caused by the afteraction following the inflation. If oxygen is used instead of air the same phenomena follow the inflation but dyspnoea fails to appear in spite of the smallness of the inspirations, and the afteraction continues to make itself felt undisturbed by dyspnoeic changes. Under otherwise similar conditions the afteraction produced by a momentary inflation of the lungs with oxygen, air or hydrogen is the same in kind and degree though not in duration.

Thus a momentary inflation of the lungs produces a depressing effect on the inspiratory activity of the centre not only during the time during which the lungs remain distended, but even after they have returned to their normal volume. We now see how such stimuli succeeding one another at a definite rate could undergo summation.

Such afteraction is not confined to a momentary inflation but also appears after a momentary diminution in the volume of the lungs. On Curve ix, Plate II the strong inspiration caused by the momentary suction is followed by an elongation of the inspiratory muscles. But the elongation has scarcely reached the crests of the normal inspirations before it becomes slow. Long before the muscles are completely elongated a second inspiration appears. This inspiration is followed by an elongation which though stronger than its predecessor is not complete. Thus each succeeding inspiration starts from a lower base line but 4—5 sec. elapse before the breathing becomes normal. During the first few seconds after a momentary diminution in the volume of the lungs the expiratory elongations are incomplete, the pause is absent and there is a distinct inspiratory tone—all signs that the centre is still under the influence of the diminution in volume.

Thus both momentary increase and diminution in the volume of

the lungs affect the respiratory centre, not only during the duration of the mechanical change of volume, but even after the lungs have returned to their normal condition.

C. The negative afteraction following increase and diminution in the volume of the lungs.

If the lungs are inflated the expiratory pause produced by the inflation is finally broken by an inspiratory contraction although the lungs are still dilated. This contraction is strong, of comparatively short duration and traces a curve with an extremely sharp crest. But if the lungs are allowed to return to the normal volume just before this interrupting inspiration would normally have made its appearance, the breathing undergoes a very different modification (Curve vi, Plate II). At the moment of collapse the inspiratory muscles contract strongly but somewhat slowly and produce a strong flat-topped curve. This contraction is of about the same strength as the interrupting inspiration but exceeds it greatly in duration. Thus sudden return of the lungs to the normal volume after an inflation of considerable duration produces a strong and long inspiratory contraction.

It might be objected that both the interrupting inspiration and the strong inspiratory effect which follows collapse after an inflation were due to the dyspnoea which must necessarily result during such a long pause in the breathing. However I think that this explanation will scarcely suffice to explain either phenomenon. For, provided the inflations are of the same strength, the pause is broken at almost exactly the same moment whether oxygen, air or hydrogen be used to inflate the lungs. It is true that the strength of the interrupting contraction is generally greatest when the lungs have been inflated with hydrogen, but the time of its appearance is the same with all three gases under otherwise similar conditions. Again the fact that the animal is breathing oxygen during and after the inflation does not diminish the strength of the inspiratory contraction which is produced by the sudden return of the lungs to their normal volume after the inflation. Indeed it is rather favourable, than otherwise, to its appearance, for if the animal is dyspnoeic this inspiratory contraction is of much shorter duration and is much more difficult to produce than when the lungs have been inflated with air or with oxygen. Thus the continued action of an inhibitory stimulus would appear to be favourable to the appearance of a burst of inspiratory energy at the moment

when the inhibition is removed. I shall call this phenomenon the negative afteraction for want of a better name.

When the inflation has not lasted very long, return of the lungs to the normal volume is often followed by a slow inspiratory contraction of great duration but of comparatively small strength (Curve iii, Plate II). Although the contraction may last 3 sec. the curve scarcely reaches the summits of the normal inspiratory curves. The elongation which follows is very incomplete, and a varying amount of tonic inspiratory contraction is present. The normal breathing is not regained until after several inspirations. It appears as if the return of the lungs to the normal volume after an inflation, which lasts some little time, tends to throw the centre into a condition particularly favourable to the appearance of inspiratory tone and favours the abolition of rhythmic breathing. From this condition of tonic activity, the centre is awakened by the inhibitory stimulus produced by the dilatation of the lungs.

As I pointed out above, collapse of the lungs to a volume below the normal is particularly active when it follows an inflation. On Curve v, Plate II complete collapse of one lung after an inflation abolished rhythmic breathing for 7 secs. Even if but a little air is allowed to enter one side of the thorax (Curve xi, Plate I), collapse of the lungs after an inflation causes an inspiratory contraction of very great duration. Now during the normal breathing of an animal with slight pneumothorax, the diaphragm is in a condition of slight tonic contraction. If we wait until the tone has disappeared, and then inflate the lungs, not only is rhythmic breathing abolished for the first few secs. after the collapse of the lungs, but even after the animal again breathes rhythmically, we notice that the tone has returned. It again disappears after a minute or more to be again recalled by another inflation.

Thus a prolonged inflation of the lungs tends, on its removal, to throw the centre into a condition favourable to the appearance of a tonic inspiratory contraction and to the abolition of rhythmic breathing.

We might expect that if a prolonged inflation of the lungs produced an inspiratory negative afteraction, a prolonged diminution in their volume might be followed by a negative afteraction, which appeared as a decrease in the inspiratory activity of the centre. Under certain conditions this is actually the case. If suction is applied and care be taken that the lungs do not dilate again to any considerable extent, a strong inspiratory contraction is produced which often lasts many seconds before the first elongation appears. If the trachea is suddenly opened before the appearance of this expiratory elongation, the lungs dilate and

under certain circumstances the inspiratory muscles elongate at once. The pause which now follows is longer than normal, and there appear to be at any rate traces of an expiratory afteraction. However this negative afteraction is by no means so satisfactory as that which follows a prolonged inflation. For it is not at all easy by means of suction to produce a prolonged inspiratory contraction in any way comparable to the prolonged pause following an inflation. When the suction has lasted 3 or 4 seconds the rhythmic breathing begins again, and all chance of a negative afteraction is past. It is true that collapse of the lungs to a volume below the normal produces a prolonged inspiratory tetanus comparable to the long pause produced by inflation, but unfortunately we are unable to bring them back to their normal volume without actually inflating them. If however a large dose of chloral has been administered, or if the brain has been separated from the medulla, the negative afteraction is often very well marked (Curve vii, Plate II); and if the animal has been experimented on for a considerable time, the strong inspiration which follows suction is frequently followed by a prolonged expiratory pause, when the lungs return to their normal volume.

To sum up the results of the experiments described in this section—

(1) Increase in the volume of the lungs causes a cessation of all inspiratory movements whether rhythmic or tonic. The expiratory pause thus produced is always finally broken by a strong inspiration provided the lungs remain long enough in a dilated condition.

Decrease in volume causes an increase in the inspiratory activity of the centre, which shows itself in the development of a strong tonic inspiratory contraction.

(2) An inflation of the lungs of short duration not only depresses the inspiratory activity of the centre during the time it lasts but also influences the breathing after the lungs have returned to their normal volume.

Momentary diminution in the volume of the lungs not only causes a strong inspiratory contraction during the time that it lasts, but also causes an inspiratory tone to appear between the rhythmic inspirations which follow the return of the lungs to their normal volume.

This has been called the positive afteraction.

(3) Provided the inhibitory pause has not been interrupted by an inspiration, return of the lungs to their normal volume after a prolonged inflation causes an outburst of inspiratory energy which tends to abolish the normal rhythm and substitute tonic inspiratory innervation.

Under certain circumstances the return of the lungs after a diminution in their volume tends to increase the expiratory phase of respiration. This has been called the negative afteraction.

(4) The essential factors of all these phenomena are independent of the gas with which the lungs may be filled. However, dyspnoea is distinctly unfavourable to the appearance of tonic innervation of the inspiratory muscles, and decidedly favourable to the maintenance of rhythmic respiration.

§ 6. *On certain other Stimuli which affect the Activity of the Respiratory Centre.*

Besides the so-called "natural" stimulation of the vagi it is of course possible to stimulate the trunks of one or both nerves electrically. Rosenthal[1] states that electrical stimulation of the divided vagi caused an increase in the inspiratory rhythm, and if strong enough might lead to an actual tetanus of the inspiratory muscles. Expiration could only follow stimulation of the vagi when the current escaped onto the superior laryngeal nerve. But if we divide one vagus and stimulate the central end with the weakest interrupted current that in any way affects the breathing, a distinct expiratory effect is produced. The pauses between the inspiratory contractions become longer than normal and the inspirations themselves are generally less powerful. As Knoll[2] has shown this is just the effect which is produced when the divided nerve is stimulated by closing its own current. If the central end of the divided vagus is dropped into the wound in the neck or onto any moist surface the inspirations are diminished in height and the pauses which separate them increased in length. The stimulus rapidly decreases in intensity but can be renewed by raising the vagus and allowing it again to fall back onto the moist surface.

With the ordinary du Bois induction apparatus, this primary expiratory effect is not always easy to see, for we rapidly pass into a region where the stimulation produces an inspiratory effect. A slight increase in the strength of the stimulus then produces the well-known "tetanus" of Rosenthal. This "tetanus" is however not produced so much by a quickening in the inspiratory rhythm as by a decrease in the completeness of the expiratory elongations (cf. Curve xiii, Plate II). The breathing is but slightly increased in rate, but with every increase in

[1] *Die Athembewegungen und ihre Beziehung zum Nervus Vagus*, Berlin, 1862.
[2] Knoll. Zweite Mittheilung. *Sitzb. d. k. Akad. d. Wissensch.* Bd. LXXXVI.

the strength of the stimulus the muscles elongate less completely until the diaphragm finally falls into a condition of tonic contraction. A further increase in the strength of the stimulus leads to the appearance of active expiratory contractions of the abdominal muscles (Curve xii, Plate II). This effect is possibly produced by the escape of the current onto the superior laryngeal nerve.

Now it is interesting to notice that the behaviour of the vagus during electrical stimulation of varying strength is very similar to that of an efferent nerve containing two kinds of fibres. Biedermann[1] has shown that the nerve which supplies the claw of the crayfish is composed of two kinds of fibres different in structure and in their behaviour to reagents. The smallest effective stimulus to this nerve causes the claw to open, whilst a slight increase in the strength of the stimulus causes it to close promptly.

If one vagus is divided and the central end stimulated electrically it is possible to regulate the stimulus so that it just fails to produce a complete "tetanus." The diaphragm remains to a certain extent permanently contracted but the rhythmic movements are not completely abolished. If the lungs are inflated during such a stimulation the diaphragm at once elongates (Curve iv, Plate II). But as soon as the lungs return to the normal volume the muscles contract violently to a considerably greater extent than before the inflation and rhythmic respiration is completely abolished for several secs. Thus an electrical stimulus, which was unable to produce a complete tetanus, is able to entirely abolish rhythmic breathing after an inflation, in consequence of the negative afteraction.

If both vagi are divided and the electrical stimulus is increased until it produces an active expiration, cessation of the stimulation is followed by a very long inspiratory contraction (Curve xii, Plate II), similar to that seen on dividing both vagi in a normal animal. Here we have an instance of negative afteraction produced by electrical stimulation when both vagi are divided. When only one vagus has been divided this same phenomenon appears but in a much less marked form owing to the restraining effect produced by the dilatation of the lung of that side on which the vagus is intact.

The changes in respiration which normally follow death from chloroform are generally said to be due to stimulation of the ends of the vagi in the lungs by the chloroform vapour[2]. But I think

[1] "Ueber d. Innervation der Krebsschere." *Sitzb. d. k. Akad. d. Wissenschaft.* Bd. xcvii. Abth. iii. März 1888.

[2] Cf. Knoll.

that the following experiment points to a rather different conclusion. If the lungs of a normal animal are inflated with either chloroform or ether vapour an expiratory pause is produced as usual. But this pause is rapidly broken by a strong and long inspiration resembling that seen when the vagi are divided. Although the lungs remain dilated the muscular strips of the diaphragm trace curves closely resembling those seen after dividing the vagi. As soon as the trachea is opened the breathing gradually reassumes the normal type. It would seem from this experiment that the chloroform or ether vapour tended rather to paralyse than to stimulate the ends of the vagi in the lungs. Again it is singularly difficult to produce the usual results which follow increase and diminution in the volume of the lungs if the animal is breathing ether or chloroform, and it would seem as if both chloroform and ether tended to anaesthetize the end organs of the vagi. However owing to the complicated action of both chloroform and ether it is very difficult to assure oneself of the truth of this theory.

The application of chloroform vapour to the nasal mucous membrane of a rabbit acts, as Kratchmer[1] showed, as an expiratory stimulation. The expiratory pauses become very long, the inspirations are weak, and if the stimulus is strong, active expirations may make their appearance. It is interesting to notice that the duration of the effect produced depends very much on whether the vagi are intact or not. In a normal animal the passage of a slight amount of chloroform vapour up one nostril caused an expiratory pause lasting 5 secs. One vagus was then divided and as nearly as possible the same stimulus caused a pause lasting 8 secs. Lastly the second vagus was divided and the pause was found to be increased to 15 secs. This experiment illustrates the effect produced by the vagi during normal respiration.

Electrical or mechanical stimulation of any ordinary sensory nerve produces an inspiratory change in the breathing[2]. But if the stomach or any of the abdominal viscera be stimulated mechanically or electrically the general result is either an increase in the expiratory pauses or more often a strong active expiration. Such an active expiration does not of course show itself on my curves except indirectly, but it is perfectly easy to assure oneself of its presence by watching the movements of the abdominal muscles and of the contents of the abdomen.

To sum up the results obtained from the experiments in this section—

[1] *Ueber Reflexe v. d. Nasenschleimhaut auf Athmung und Kreislauf*, 1870.
[2] Knoll.

(1) Electrical stimulation of the divided vagus produces *firstly* an increase in the expiratory pauses and a decrease in the strength of the inspiratory contractions; *secondly* Rosenthal's "tetanus" which is due rather to incomplete expiratory elongation of the inspiratory muscles than to an increase in the rapidity of the inspirations. And *thirdly*, probably owing to escape of current, active expirations accompanied by movements of swallowing.

(2) The application of chloroform vapour to the nasal mucous membrane causes expiratory pauses the length of which are very dependent on whether the vagi are intact or not.

(3) Stimulation of any sensory nerve in the body wall produces an inspiratory effect, whereas stimulation of the visceral sensory nerves tends to cause an active expiration.

§ 7. *Ventilation or "Artificial Respiration".*

We have examined the effect of a single diminution or increase in the volume of the lungs and now pass on to the phenomena which follow their periodic repetition. Most of the machines for "Artificial Respiration" or "Ventilation" simply inflate the lungs periodically and allow them to return to the normal volume after each stroke of the pump. With all such apparatus, it is only possible to watch the effect of periodically repeating short inflations. But in 1870 Prof. Hering designed a double pump by which both periodic increase and periodic decrease in the volume of the lungs could be produced. When the whole pump is in action the first stroke pumps a certain quantity of air or other gas into the lungs and the next stroke removes exactly the same quantity. The strength and rate of the inflations and suctions can be regulated with the greatest nicety. Now it is obvious that by connecting one half only of the pump with the trachea it is possible to produce periodic inflations, whilst by using only the other half the ventilation consists simply of periodic suctions. Under such circumstances each inflation or each suction as the case may be is separated from the next by a nicely regulated pause during which the lungs can return to their normal volume. I have found it convenient to speak of ventilation which consists only of periodic inflations as positive; that consisting of periodic suctions as negative; and that consisting of alternate suctions and inflations as compound.

If the first inflation of positive ventilation happens to fall in the inspiratory phase the inspiratory contraction is at once cut short.

As soon as the first stroke of the pump is over another inspiration begins and may reach its full height before the occurrence of the next inflation. Thus the normal rhythm quickly adapts itself to the periodic stimulation and each stroke of the pump corresponds to a pause in the breathing, each pause in ventilation to an inspiratory contraction. The breathing now "follows" the artificial respiration.

But when discussing the effect of a single short inflation I pointed out that every inflation exerted a depressing influence on the inspiratory activity of the centre even after the lungs had returned to their normal volume. Thus every stroke of the pump during positive ventilation not only produces an expiratory pause during the time that it lasts but also tends to decrease the size of the following inspiratory contraction. During positive ventilation every inspiration is smaller than that which preceded it. The inspiratory contractions get smaller with every stroke of the pump until they entirely disappear (Curve vii, Plate V). See also *b.* woodcut, p. 31. The muscles then remain quiescent even between the strokes of the pump. If ventilation is now stopped a pause follows which Rosenthal called Apnoea. But we must not forget that the cessation of all rhythmic respiration during the time that the ventilation lasts is the same phenomenon as the pause which appears on stopping the ventilation, whatever theory we may adopt to explain it. I therefore propose to call the former Apnoea of Ventilation and the latter the Apnoea pause.

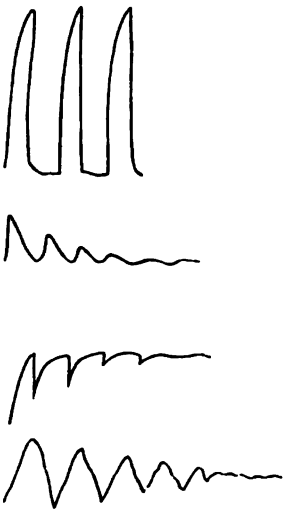
If we use the opposite form of ventilation in which each stroke of the pump sucks a given quantity of air out of the lungs the breathing is modified in a very different way (Curve vi, Plate III). The first suction causes a strong inspiratory contraction. As soon as the suction is over the lungs dilate and the inspiratory muscles elongate rapidly. Then follows the second stroke of the pump accompanied by a second strong inspiratory contraction. Here again the breathing "follows" the artificial respiration although with exactly the converse movements to those produced during periodic inflations. Here each stroke of the pump is accompanied by an inspiratory contraction and followed during the pause by an expiratory elongation. But as I showed above, each momentary suction tends not only to produce an inspiration during the time it lasts but also to depress the expiratory activity of the centre even after the lungs have returned to their normal volume. Thus every elongation is less complete than the one which preceded it, and finally all rhythmic respiratory movements are completely abolished. But although rhythmic breathing has ceased the inspiratory muscles

are contracted to an extent far in excess of any normal inspiration. This is exactly the converse phenomenon to the expiratory pause which finally results during periodic inflations and I propose to call it the Apnoea of negative Ventilation. See c. woodcut, p. 31.

Some may object to the use of the word apnoea for such a condition as this and insist that it can only be applied to a condition in which all the respiratory muscles are at rest. But there is nothing in the meaning of the word which prevents its application to the cessation of all rhythmic respiration, whether the inspiratory muscles be tonically contracted or not. The one type is so exactly the complement of the other that one generic term is required for both, and I shall therefore speak of the phenomena following negative ventilation as "Inspiratory Apnoea" and that following positive ventilation as "Expiratory or Inhibitory Apnoea". If stress is laid on the form of the ventilation independent of the form of apnoea it produces, the apnoea will be spoken of as that of positive or negative ventilation as the case may be.

As we might expect, compound ventilation produces an apnoea intermediate between the other two forms (Curve v, Plate IV). Supposing the first stroke of the pump produces an inflation the inspiratory contraction is promptly cut short. But this inflation is at once followed by a suction which calls forth a strong inspiration. Thus each complete cycle of the pump produced two distinct effects—firstly an expiratory effect and secondly an inspiratory contraction; and the breathing takes up the rhythm of the ventilation. Now each inflation tends to abolish the inspiratory rhythm of the centre even after the lungs have returned to their normal volume. Each suction tends to produce an exactly opposite afteraction. The resultant of these two opposite afteractions is very interesting and consists in the total abolition of rhythmic respiration and the substitution of a certain amount of tonic inspiratory contraction. The inspiratory contractions and the expiratory elongations become very small and finally disappear, the lever tracing a line of varying height according to the relative value of the two stimuli. For although the actual increase in the volume of the lungs under the influence of the one stroke may be exactly equal to their diminution at the next stroke, the stimuli so caused are not always of equal value. The conditions which cause one form of stimulus to be more effective than the other will be described later on.

If the curve produced by ventilation composed of alternate inflations



a. normal breathing.

b. apnoea. positive ventilation.

c. apnoea. negative ventilation.

d. compound ventilation.

and suctions is carefully examined and compared with those produced by positive and negative ventilation, the clearest traces of double action can be seen. During positive ventilation each inspiratory curve is sharply pointed and is separated from the others by a considerable interval. During negative ventilation on the other hand the crests of the inspiratory curves are broad and elongated and the expiratory periods are very short. But during compound ventilation the crest of each inspiration is separated from that of the next inspiration by almost exactly the same interval that separates the trough of each elongation curve from the lowest point of the next elongation.

If one side of the thorax is opened so that the lung of that side collapses, periodic inflations produce an apnoea closely resembling that caused by compound ventilation (Curve v, Plate VI). Each inflation causes the usual decrease in inspiratory activity, but each return of the lungs to their abnormally diminished total volume produces a strong inspiratory effect. In fact the collapse of the lungs after each inflation may now produce quite as strong an inspiratory effect as if compound ventilation were applied to the lungs of an uninjured animal. Even if but a slight amount of air has been allowed to enter one side of the thorax, positive ventilation no longer produces a complete expiratory standstill

but leaves the diaphragm in a condition of slight tonic contraction after rhythmic breathing has been abolished.

But now and then even in a perfectly normal animal positive ventilation leaves the diaphragm slightly contracted after rhythmic breathing has been abolished. If the ventilation is continued for a considerable time after rhythmic respiration has ceased, the muscles gradually elongate and may finally elongate completely. But if the ventilation is weak and the animal very sensitive traces of inspiratory tone can be discovered even after the rhythmic breathing has ceased (Curves iv and viii, Plate V). Although not a normal occurrence this phenomenon is especially prone to appear in animals in which a single inflation was followed by a strong negative afteraction.

When we consider the form assumed by the apnoea in consequence of periodic suctions, we must not forget that this form of ventilation is in reality only an extreme form of compound ventilation. For during the later stages of negative ventilation the lungs do not dilate to the normal volume after each suction but to a volume far above the normal. The diaphragm is strongly contracted even between the strokes of the pump and, as soon as each suction ceases, the lungs expand proportionately. It is indeed very doubtful whether during the later stages of negative ventilation the suctions really reduce the volume of the lungs even to the normal. Yet in spite of this each suction undoubtedly still produces an inspiratory effect. Thus even negative ventilation is only an extreme variation of compound ventilation.

Now in certain animals expansion of the lungs appears to be a particularly active stimulus. The slightest dilatation of the lungs produces a marked effect on the breathing, whilst diminution in their volume produces comparatively little result. In such animals negative ventilation produces the usual abolition of rhythmic breathing, but the tonic contraction is scarcely stronger than that produced by compound ventilation in other animals (cf. Curves i and iii, Plate VI). Here the fact that the lungs return to a volume above the normal between the strokes of the pump seems to greatly decrease the strength of the inspiratory tone which is produced by this form of ventilation.

In one animal, out of the many used in these experiments, the inspiratory tone produced by negative ventilation was not nearly as great as that caused by compound ventilation in a normal animal. A slight inflation produced a pause of quite abnormal length, and on closing of the trachea in the inspiratory phase a pause appeared as long as that normally following an inflation.

To sum up the results of this section—

(1) Periodic inflations gradually abolish rhythmic breathing and produce a pause during which the inspiratory muscles generally remain completely elongated.

(2) Periodic suctions also abolish rhythmic respiration but the inspiratory muscles remain in a condition of permanent tonic contraction.

(3) Compound ventilation produces phenomena intermediate between those which are produced by the two other forms of ventilation.

(4) If one lung is allowed to collapse positive ventilation now produces an apnoea closely resembling that following compound ventilation, and under certain circumstances even in the normal animal the disappearance of rhythmic breathing in consequence of positive ventilation may be accompanied by the appearance of a slight inspiratory tone. Conversely, negative ventilation may sometimes produce an inspiratory tone but slightly stronger than that ordinarily caused by compound ventilation.

§ 8. *The Apnoea Pause.*

All forms of ventilation produce one phenomenon in common—the abolition of rhythmic respiration—although the condition of the inspiratory muscles varies very much according to the nature of the ventilation.

We have seen that positive ventilation leads to the production of an apnoea which is normally characterised by the complete elongation of the inspiratory muscles. When the ventilation ceases the muscles remain perfectly quiescent for a varying length of time (Curve vii, Plate V). The apnoea pause is finally broken by a small and shallow inspiratory contraction. Then follows a considerable pause, which is broken by a slightly stronger inspiration. The normal type of breathing is gradually regained by an increase in the strength of the inspiratory contractions and by a decrease in the pauses between them. Such an apnoea pause is the exact continuation of the apnoea which appeared during the positive ventilation. But the longer the pause lasts, the poorer must be the condition of the air in the lungs, until finally the pause is broken by an inspiration. But the centre is still dominated by the afteraction of the ventilation and a very slight dilatation of the lungs is sufficient to check the inspiratory contractions. As the influ-

ence of the afteraction gradually wears off the inspirations increase in size and the expiratory pauses decrease in duration.. In fact the recovery of the centre after an apnoea pause is accompanied by the same phenomenon that appeared after a momentary inflation. The assumption that the pause was broken by an inspiration in consequence of the growing dyspnoea is supported by the fact that the only difference between the apnoea produced with oxygen or with air lies in the postponement of the first inspiration.

But this is not the only type of pause which follows the cessation of positive ventilation. If the ventilation is stopped as soon as the rhythmic breathing has ceased, the muscles frequently do not remain elongated (Curve iii, Plate III). They begin to contract slowly as soon as the ventilation ceases and trace a slow contraction curve which may finally reach the height of the crests of the normal inspirations. In this case the pause is replaced by a slow inspiratory contraction of great duration. This contraction is followed by an incomplete elongation of the inspiratory muscles, and the normal breathing is gradually regained by an increase in the expiratory elongations and by a slight simultaneous increase in the strength of the inspiratory contractions. I long thought that this phenomenon was due to dyspnoea but the substitution of hydrogen for air as the medium of ventilation led rather to the abolition of this gradual rise than to its increase.

If we count the number of inflations that are sufficient to produce this form of pause and then repeat the experiment, increasing the duration of the ventilation, the pause is modified. For the first few seconds after the ventilation has ceased the muscles remain relaxed and a true expiratory pause appears. But the muscles then begin to contract slowly and form the same long slow contraction curve that I have just described. If on the other hand we decrease the duration of the positive ventilation the breathing assumes a peculiar type when the ventilation ceases (Curve v, Plate III). The muscles contract quickly to about half the height of a normal inspiration and trace a long flat curve lasting about three seconds. Then follows the first elongation, which is cut short by the appearance of another flat inspiration. Each subsequent inspiration becomes stronger and each expiratory period longer until the normal type of breathing is regained. Thus if the duration of the ventilation is short and the animal sensitive a more or less tonic form of inspiratory energy seems particularly liable to invade the period which intervenes before the normal rhythm is reestablished.

I pointed out above that the apnoea which appears during negative

ventilation is the resultant of the action of two opposing forces, the diminution in volume caused by the suctions and the return to a volume, considerably exceeding the normal, after each stroke of the pump. The rhythm is abolished and the muscles remain in a condition of tonic contraction of varying strength. Now this resultant afteraction lasts even after the ventilation has ceased just as was the case with the afteraction caused by positive ventilation. As soon as the ventilation stops, the muscles begin to elongate (Curves iv and vi, Plate III). But long before they have completely elongated they remain for a moment stationary and then contract again slightly. Then follows a more complete elongation and true rhythmic respiration begins again. But both the expiratory elongations and the inspiratory contractions are feeble and regular rhythmic breathing is not reestablished until several seconds have elapsed.

If this form of apnoea pause were the exact expression of the afteraction caused by the negative ventilation the muscles would remain contracted to exactly the same extent as during the ventilation. But we must not forget that the lungs are dilated to a quite abnormal extent. The strong tonic inspiratory contraction which is produced by the afteraction tends to cause its own destruction by enormously dilating the lungs. It is therefore no wonder that the muscles begin to elongate as soon as the ventilation ceases. The point to be noticed is that rhythmic breathing fails to appear for several seconds after the ventilation has ceased in spite of the great dilatation of the lungs. Even when the rhythmic breathing reappears, it is characterised by the incomplete expiratory elongations which we have so often taken as an indication that the centre is still under an inspiratory influence. The stronger the ventilation the more gradual is the elongation of the muscles and the longer the time that elapses before rhythmic breathing is reestablished (cf. Curves iv and vi, Plate III). The following experiment tends to show that, but for the dilatation of the lungs which it produces, the inspiratory afteraction would continue to cause exactly the same tonic contraction of the inspiratory muscles even after the ventilation ceased. The right vagus is divided and the left lung allowed to collapse by puncturing the thorax. As I pointed out above positive ventilation, in an animal so treated, causes a strong inspiratory apnoea. The rhythmic breathing is abolished, but the muscles remain strongly contracted just as during negative ventilation in a normal animal. If the ventilation is stopped the centre is under the influence of a strong inspiratory afteraction. But the left lung cannot expand

under its influence, and the expansion of the right lung does not, under favourable circumstances, influence the activity of the centre for the vagus which supplies it is divided. The inspiratory afteraction is therefore able to show itself unhindered by the changes in the capacity of the thorax which it produces. The inspiratory muscles remain permanently contracted until the advent of the first feeble expiratory elongation, and the lever traces a horizontal line without any trace of the fall which was so noticeable after negative ventilation in a normal animal. In fact the curve sometimes even rises during the apnoea, probably in consequence of some influence from the collapsed lung.

I shall describe fully later the effect of dividing both vagi without stimulation at the moment when the negative ventilation ceases. But what concerns us now is that, under such circumstances, the elongation of the inspiratory muscles entirely fails to make its appearance. If the vagi are successfully divided at the moment when the ventilation is stopped, the muscles remain contracted to the same extent as during the ventilation and the appearance of rhythmic respiration is considerably deferred. (Curve iv, Plate VI.)

Thus the form assumed by the apnoea pause which follows negative ventilation depends upon the interaction of two antagonistic stimuli on the respiratory centre. (1) The afteraction which has been formed during the ventilation as the resultant of the inspiratory effect of the suctions and the expiratory effect of the return of the lungs between the strokes of the pump. And (2) the expiratory stimulus caused by the increased volume of the lungs which this afteraction tends to produce.

The cessation of compound ventilation is followed by a pause of considerable length. The muscles elongate slowly to a slight extent with even decreasing rapidity. Finally they contract again slowly, and then a quick but incomplete elongation marks the return of rhythmic respiration (Curve iii, Plate VI). The amount to which the muscles elongate during the apnoea pause is inversely as the amount of tonic contraction produced by the ventilation. If the ventilation causes an apnoea more nearly approaching the inspiratory form the elongation is considerable; if on the other hand the tonic contraction produced by the compound ventilation is comparatively small the curve traced by the apnoea pause is an almost complete continuation of the curve of ventilation. But in every case the fall during the apnoea is less abrupt than during the apnoea caused by negative ventilation in the same

animal. This is probably due to the small extent to which the lungs are dilated by the afteraction of this form of ventilation compared with the dilation caused by the afteraction of negative ventilation. The inhibition from the lungs is therefore weaker and the muscles elongate more slowly.

The explanation I have given for the various forms assumed by the apnoea pause rests upon the assumption that the centre can be influenced by stimuli from the lungs even during apnoea. But Rosenthal[1] states that stimulation (electrical) of the vagi is entirely without effect during apnoea. In their first communication Hering and Breuer[2] make the same statement, but in their second paper they show that collapse of the lungs causes an inspiration even during apnoea, provided it be not too deep.

If the lungs are inflated during an inspiratory apnoea the inspiratory muscles elongate rapidly and remain completely quiescent during the duration of the inflation (Curve i, Plate IV). In fact this form of stimulation appears to be particularly active during an inspiratory apnoea, to judge by the length of time which elapses before the pause is broken by an "interrupting" inspiration. If this inflation is of short duration the muscles elongate as before but contract again strongly as soon as the inflation is over (Curve iv, Plate VII). This contraction is terminated by a slight elongation, which is quickly followed by another contraction, so that for many seconds the rhythmic respiratory movements oscillate about a raised base line. Thus the production of an inflation during an inspiratory apnoea tends to increase those phenomena which I described above under the term of negative afteraction.

It is obvious that an inflation can cause no additional elongation during a purely inhibitory apnoea, caused by positive ventilation. But after positive ventilation, the period, which intervenes before the rhythmic breathing is reestablished, is frequently occupied by a slow contraction of the inspiratory muscles (vide p. 34). In this case an inflation at once cuts short the contraction and the muscles remain in a condition of complete elongation until the pause is broken by a strong "interrupting" inspiration (Curve ii, Plate III) such as normally terminates the pause caused by an inflation. Thus we see that this gradual rise can be stopped by applying a stimulus of the same kind

[1] Cap. 8. *Die Athembewegungen u. ihre Beziehung z. Vagus.*
[2] p. 926. *Sitzb. d. k. Akad. d. Wissensch.* Abth. ii. 1868.

as the afteraction of the ventilation. A short inflation also causes an elongation, but as soon as it is over the muscles contract to a point considerably above the level they had reached when the inflation began. Thus whilst the actual inflation annihilates the slow contraction in this form of apnoea the negative afteraction of an inflation distinctly increases it.

If the lungs are diminished in volume during a complete expiratory apnoea following positive ventilation an enormous inspiratory contraction is produced. This inspiration is both stronger and of longer duration than any inspiration after the vagi have been divided. It is also noticeable that the inspiration produced by a suction applied during this form of apnoea is of much greater duration than when the suction is applied during normal breathing. Diminution in the volume of the lungs seems to act more effectively, to judge by the strength and duration of the contraction it produces, when the centre has been made apnoeic by periodic inflations. If the muscles are slowly contracting during the apnoea, diminution in the volume of the lungs hastens and enormously increases the contraction (Curve i, Plate III). If the suction is of short duration ("momentary") the muscles contract rapidly but do not elongate completely after the suction is over (Curve iii, Plate VII). The cessation of the suction is followed by a slow elongation of the inspiratory muscles, and even when the rhythmic breathing begins again, an inspiratory tone is distinctly present. This effect is probably due to the positive afteraction of the momentary diminution in the volume of the lungs.

Thus we find that, whatever form the apnoea pause may assume after positive ventilation, it is greatly modified by a diminution in the volume of the lungs. It would appear as if the centre were particularly sensitive to this form of stimulation when under the influence of an apnoea caused by periodic inflations.

Diminution in the volume of the lungs, whether momentary or of considerable duration, also greatly modifies the form assumed by an inspiratory apnoea (Curve iv, Plate IV). If the muscles have begun to elongate when the suction is applied, they contract again and trace an even stronger curve than during the ventilation.

When treating of the form which the pause assumed after negative ventilation, I explained the fall in the curve as due to the inhibitions caused by the dilatation of the lungs. Here we have removed the influence of this dilation by diminishing the volume

of the lungs and the curve therefore rises again. The inspiratory after-action has been strengthened by the removal of the inhibition from the dilated lungs which hampered its activity.

It is scarcely necessary to add that both increase and diminution in the volume of the lungs greatly alter the apnoea pause produced by compound ventilation. In fact this form of apnoea pause acts in every way like an apnoea produced by negative ventilation in which the tonic inspiratory contraction is feeble.

To sum up the results of the experiments just described—

(1) The apnoea pause which follows periodic inflations of the lungs may vary between two extreme types. It may either consist of a pause during which the inspiratory muscles are completely relaxed; or the period which elapses between the close of ventilation and the beginning of rhythmic breathing may be occupied by a slow contraction of the inspiratory muscles.

(2) The apnoea produced by negative ventilation consists of a slow elongation of the tonically contracted inspiratory muscles; but even when rhythmic breathing begins again a strong inspiratory tone is still present.

(3) Compound ventilation produces a similar apnoea pause which is of longer duration and is characterised by less tonic contraction than that produced by negative ventilation.

(4) Both increase and diminution in the volume of the lungs are active stimuli during all forms of apnoea, provided the ventilation has not lasted too long.

(5) The various forms assumed by the apnoea pause under different conditions are due to the modifying influence which the position of the lungs exerts on the afteraction produced by the various forms of ventilation.

Note. In all these experiments I have only applied Artificial Respiration (Ventilation) for a very short time. For the experiments I have so far described it was not necessary to continue it after rhythmic respiration had been abolished. But I must here add a warning against the evil effects produced by long-continued positive ventilation. Positive ventilation causes a considerable fall in the blood-pressure and also great loss of animal heat if continued for long. After about ten minutes of positive ventilation the centre no longer acts with the same promptness to various stimuli, and even half-an-hour after the ventilation has ceased it is apparently still enfeebled. (Cf. Knoll who states that long-continued ventilation may even cause anaemia of the brain.) In every case where it is necessary to apply artificial respiration for long, it should consist of alternating suctions and inflations.

§ 9. *The Effect produced by the Gas used in Ventilation on the form assumed by the Apnoea.*

So far artificial respiration has been treated purely as the periodic repetition of certain mechanical stimuli. We have watched the gradual development of the apnoea under the influence of each stroke of the pump and we noticed how the form assumed by the pause depended on the character of the ventilation and on the particular volume assumed by the lungs. It is thus extremely improbable that the increased aeration of the blood is the cause of the phenomena of apnoea in the normal animal. For the varying forms of apnoea are so exactly what we should expect from the study of the results produced by a single inflation or collapse of the lungs that we are compelled to explain them as the result of summation of rhythmic stimulations. Yet on the other hand the constitution of the air in the lungs does play a very important part in the production of apnoea even when the vagi are intact.

If the lungs are inflated with hydrogen or with oxygen, the result is in all cases the same. The only difference in the result produced by a momentary inflation lay in the duration of the afteraction. Now the two gases play exactly the same part when the stimulus is periodically repeated. If the lungs are periodically inflated with pure hydrogen, each stroke of the pump tends to decrease the inspiratory activity of the centre. The inspirations get gradually smaller, and finally all rhythmic movements disappear and a true apnoea of ventilation is produced just as if the lungs had been periodically inflated with air. If the ventilation is stopped a true apnoea pause is sometimes, though rarely, seen. More frequently rhythmic breathing begins again as soon as ventilation ceases although in a greatly modified form (Curve vi, Plate V). The first inspiration is very small and is followed by a considerable pause. The next is somewhat stronger and the contractions increase in strength until they reach a height above that of the normal inspirations. The breathing has become distinctly dyspnoeic for the inspirations do not last longer than the normal and each is followed by a complete pause. We have thus succeeded in producing the complete abolition of the respiratory rhythm by periodically inflating the lungs with hydrogen. On ceasing the ventilation the dyspnoea is so great that the breathing frequently recommences although the inspiratory activity is distinctly checked by the afteraction of the ventilation. As this afteraction is gradually overcome the inspirations assume the

normal dyspnoeic type—they are strong but of comparatively short duration and are not accompanied by the presence of an inspiratory tone. Now it is interesting to notice that the curve traced by the gradual development of the normal breathing after ventilation with hydrogen very closely resembles that produced by the gradual development of rhythmic breathing after an inhibitory apnoea pause (cf. Curves vi and vii, Plate V).

If ventilation with hydrogen is continued after the rhythmic breathing has ceased, the dyspnoea becomes so strong that the inspirations begin again during the ventilation. Rhythmic breathing reappears in exactly the reverse order to that in which it was abolished. The first inspiration is very small and is followed by a pause which corresponds to a stroke of the pump. As soon as this inflation is over a slightly stronger inspiration makes its appearance. On the curve which I have chosen to illustrate this phenomenon (Curve v, Plate V), the ventilation was stopped after the fourth inspiration, and the dyspnoea now appeared in full force unchecked by the ventilation. The inspiration which follows the cessation of ventilation is nearly five times as strong as its predecessor. The inspirations continue to increase in strength until the lungs have been freed from hydrogen. The expiratory pauses are however quite as long and sometimes longer than the normal and the inspiratory contractions are of comparatively short duration compared with their strength.

I had hoped to be able to show that negative ventilation could also produce an apnoea when the lungs were filled with hydrogen. But the technical difficulties were so great that we must be content with but an indirect proof. I pointed out above that negative ventilation was in reality only an extreme form of compound ventilation, and that the inspiratory apnoea produced by compound ventilation is only an inspiratory apnoea weakened by the inflations which alternate with the suctions. Now it is perfectly easy to use hydrogen as the medium for compound ventilation. The first stroke of the pump inflates the lungs with hydrogen and the second stroke removes the same amount of the mixed gases in the lungs. After the first few strokes the atmosphere in the lungs must consist, if not entirely at any rate to a very large extent, of hydrogen. Now not only does this form of ventilation abolish all rhythmic respiratory movements and produce the usual inspiratory apnoea but cessation of ventilation is generally ever followed by a considerable apnoea pause (Curve vi, Plate IV). In a normal animal 14 double strokes of the pump produced an apnoea

pause of 16 secs. when air was used (Curve v, Plate IV). The same number of strokes of the pump with hydrogen also produced a distinct pause lasting six secs. from which the normal breathing is regained as usual (Curve vi, Plate IV).

Thus the substitution of hydrogen for air as the medium of inflation does not materially alter the appearance of the inspiratory apnoea produced by compound ventilation. Now the tonic inspiratory contraction is produced by the periodic suctions which alternate with the inflations, and as the former still produce the normal effect although the lungs are filled with hydrogen there is no reason for supposing that negative ventilation would not produce an apnoea under the same conditions provided we were able to apply it.

The substitution of oxygen for air as the medium of ventilation causes but slight changes. I showed in a previous section, when discussing short inflations, that oxygen postpones the advent of dyspnoea and so allows the afteraction of the inflation to show itself for a longer time than would otherwise have been possible. When ventilation is carried on with oxygen the rhythmic breathing is not abolished any more rapidly than normal; the same number of strokes of the pump are required to annihilate the inspirations with oxygen as with air (cf. Curves ii and iii, Plate IV). The pause which follows the cessation of ventilation is exactly of the same nature whichever gas has been used, though it tends to last longer when the lungs have been periodically inflated with oxygen (cf. Curves iv and viii, Plate V). But not only does the pause last longer but we also find that the breathing returns more slowly to the normal type than if air had been used. This would seem to show that even when the apnoea pause has been broken by a rhythmic respiratory movement, the afteraction of the ventilation is only weakened, not exhausted. Otherwise it is difficult to understand why the return to the normal type should be delayed, when dyspnoea is prevented by filling the lungs with oxygen.

In the same way oxygen does not alter the phenomena produced by compound ventilation. Rhythmic breathing disappears at the same rate and the tonic inspiratory contraction is of the same strength as if air had been used. The only difference lies in the slightly increased length of the apnoea pause.

I at one time thought that the long slow contraction of the inspiratory muscles which sometimes occupies the period of apnoea after positive ventilation might be due to a struggle between the afteraction and dyspnoea. However on substituting hydrogen for air I found that

the apnoea was shorter and that the rise was generally less than when air was used (cf. Curves i and ii, Plate V). Oxygen seemed neither to increase nor decrease this form of apnoea. The muscles began to contract as soon as the ventilation ceased and, as soon as the curve reached a certain height, the muscles elongated as usual and the normal breathing was regained in the usual manner. Thus this form of apnoea cannot be due to the struggle between dyspnoea and the afteraction of the ventilation but is probably produced by some condition of the centre perhaps analogous to the negative afteraction which sometimes follows a single inflation.

To sum up the experiments in this section—

(1) Periodic inflations with pure hydrogen abolish rhythmic breathing as usual. An actual apnoea pause of short duration may appear when the ventilation ceases but more often the rhythmic breathing begins again at once. In the latter case the first inspirations are small and the contractions gather strength until the breathing assumes a distinctly dyspnoeic type.

(2) Compound ventilation with hydrogen produces the normal results except that the pause is considerably shorter than if air is the medium of inflation.

(3) The substitution of oxygen for air in either positive or compound ventilation increases the length of the apnoea pause but in no way hastens the abolition of normal rhythmic breathing.

(4) The constitution of the atmosphere in the lungs does not determine the form assumed by the apnoea in a normal animal. This apnoea is due to the summation of the stimuli caused by the periodic alterations in the volume of the lungs. Dyspnoea tends to cut short the appearance of this afteraction by causing the reappearance of rhythmic breathing. Thus oxygen increases the length of the apnoea by averting dyspnoea and hydrogen shortens the pause owing to the dyspnoea which it causes.

§ 10. *Apnoea after Division of the Vagi.*

Rosenthal in his well-known work on the nervous mechanism of respiration stated that apnoea was due to an over oxydation of the blood which put the centre to rest by removing its normal stimulus. But of late years several physiologists have insisted on the part played by the vagi in the production of apnoea in the normal animal. Meischer-Rüsch[1] distinguished an "apnoea vera and an apnoea vagi"; but to the

[1] du Bois Reymond's *Archiv*, Heft 5 and 6, 1885.

best of my knowledge he did not point out how they differed from one another. Knoll[1] found that it was generally impossible to produce an apnoea after the vagi had been divided, and states that a true apnoea can only be produced when the vagi are intact. However he found that the breathing was in all cases weakened and flattened by ventilation after division of the vagi, and that he could on two occasions produce a real apnoea pause. But he dismisses these apparent exceptions with the remark that apnoea only occurs, after division of the vagi, when the irritability of the centre is diminished owing either to the narcotic or to the fact that the animal has been experimented on for a long time. But it is *a priori* by no means improbable that the very fact that the vagi are divided tends either directly or indirectly to diminish the irritability of the respiratory centre.

There is however one fundamental difference between the behaviour of the centre before and after division of the vagi. As I pointed out in the previous section ventilation with hydrogen is able to abolish rhythmic respiration in the normal animal. Now if the vagi are divided the only effect of ventilation with hydrogen is the production of a violent dyspnoea. The inspirations get gradually stronger as ventilation continues, and finally the experiment is ended by violent movements of the whole animal. There is not a trace of the gradual abolition of rhythmic breathing which always follows the first few strokes of the pump in the normal animal.

But if air is used to periodically inflate the lungs both inspiratory contractions and expiratory elongations decrease in strength. The inspiratory tone which is generally present after the vagi have been divided grows stronger, owing to the decrease in the expiratory elongations, and the inspirations become shallow. But the breathing does not "follow" the strokes of the pump, as was the case before the vagi were divided; the respiratory movements remain entirely independent of the rhythm of ventilation although they gradually decrease in strength. After some 40 secs. of rapid ventilation this is frequently the only result produced. But under certain circumstances both inspirations and expirations decrease in strength until the rhythm disappears, leaving the diaphragm in a condition of greater or less tonic contraction (Curve i, Plate IX). After the close of ventilation the muscles remain quiescent for a varying time, producing an apnoea pause which is characterised by a varying amount of inspiratory tone. The rhythmic breathing may then

[1] Mittheilung III. *Ueber Apnoe.*

be reestablished by a gradual increase in the strength of the inspirations and expirations. In fact the centre sometimes recovers from the apnoea by exactly the reverse stages to those by which it became apnoeic. There is scarcely room for doubt that the muscles are really strongly contracted during the apnoea pause after examining Curve i, Plate IX, but in order to be assured of the fact I divided the phrenic nerves during a similar though somewhat stronger apnoea pause. The point of the lever at once fell 4·5 c.m., showing that the tonic contraction was very considerable.

The longer the vagi have been divided the easier it becomes to produce an apnoea and the weaker is the tonic contraction that appears during the pause. We can watch the gradual decrease in the tone by resorting to apnoea every five minutes after dividing the vagi. The greatest number of strokes of the pump are required immediately after division, but the tone is then very strong. As time advances fewer strokes suffice, but the tone is much weaker. In fact we often find that, as time goes on, it gradually becomes possible to produce an apnoea even in those animals in which ventilation failed to completely abolish rhythmic breathing immediately after the vagi were divided. The normal breathing is now often regained in a somewhat different manner to that described above. After the pause has lasted a variable time the lever begins to rise slowly. This contraction of the inspiratory muscles is extremely gradual and may last as long as half a minute. When the curve has reached the height of an ordinary inspiration (after division of the vagi) the muscles elongate quickly. This elongation is often incomplete, and we frequently find that the tonic contraction which may have disappeared before the ventilation was begun has now reappeared. But even when the tone does not reappear it is noticeable that the inspiratory contractions which follow the apnoea last considerably longer, though they may not be stronger than those which preceded the ventilation.

The method by which rhythmic breathing is reestablished after an apnoea, when the vagi are divided, is very variable and may present many modifications of the two extreme types that I have described.

It is scarcely necessary to add that ventilation consisting of periodic suctions and inflations (compound) produces an apnoea in every case where it can be produced by periodic inflations. But it is interesting to notice that in those cases where an apnoea can be produced with ease negative ventilation is also sometimes able to abolish the rhythmic breathing. The apnoea is however in all cases the same whatever

the ventilation may be. The nature of the ventilation has no influence on the form assumed by the apnoea as was the case before the vagi were divided.

How are we to explain the occurrence of apnoea after division of the vagi? It is probably not due to the stimulation of other sensory nerves, for the respiratory movements which occur during the time ventilation is being carried on do not bear any relation to the strokes of the pump. Again, it is possible to produce an apnoea if the greater part of the wall of the thorax be removed and if the spinal cord has been divided between the sixth and seventh cervical vertebra. It appears to bear the most distinct relation to the condition of the air in the lungs, and yet after the recent researches of Gad and others we are scarcely justified in assuming that it is due to the over oxydation of the blood. In order to show that the blood was not overloaded with oxygen during the apnoea Gad[1] devised the following simple experiment. He cut away part of the chest wall of a rabbit in the middle line and watched the colour of the heart during normal respiration. This can be easily done in a rabbit, for the anterior mediastinum is so large that a considerable opening can be made in the chest wall without causing the lungs to collapse Ventilation was now begun and an apnoea produced. Now Gad noticed that even during complete apnoea the right side of the heart was darkly venous, showing that at any rate one half of the blood was not overloaded with oxygen. But as far as I can gather the vagi were still intact in the animal on which he made this observation and the apnoea he produced was therefore probably a vagus apnoea. I therefore repeated his experiment after dividing the vagi. If some time has elapsed since the vagi were divided the blood on the left side of the heart appears to be somewhat darker than in a normal animal. A few seconds after ventilation begins the left side of the heart gets distinctly redder, and just before the rhythmic breathing ceases the heart appears to have assumed its normal bright colour. The right side of the heart however remains dark coloured, even at the end of ventilation. If ventilation is now stopped the left side of the heart gets steadily darker during the apnoea pause. Just before the first rhythmic movement appears the blood on the left side of the heart appears to have reached a quite abnormal degree of venocity. The results of the experiment render it difficult to assume that the blood in the whole system is overcharged with oxygen during the apnoea, for

[1] *Apnoe*, p. 3, Würzburg, 1880.

even at the height of ventilation the right side of the heart still remains venous. It would appear as if the blood on the left side of the heart was permanently below the normal degree of oxydation owing to the inefficient respiratory movements which follow division of the vagi. Now if we suppose that the respiratory centre gradually adapts itself to this dyspnoeic mixture it is easy to see how ventilation could produce an apnoea. Each stroke of the pump improves the constitution of the atmosphere in the lungs and thus enables the blood to take up an increased amount of oxygen until it even reaches its normal oxydation. But the centre is adapted to a mixture containing less oxygen and thus even normal blood will now appear hyperarterialised. If the ventilation is now stopped the atmosphere in the lungs is able to supply the blood with oxygen during several journeys round the system, for rhythmic breathing need not begin until the air in the lungs has become distinctly dyspnoeic. In addition to this we must bear in mind that division of the vagi possibly directly lowers the irritability of the respiratory centre. According to the view I have just put forward the blood is not overloaded with oxygen during apnoea following division of the vagi; but partly owing to direct diminution in the vitality of the centre and partly owing to the fact that it has become adapted to a dyspnoeic condition of the blood an increase in the oxygen of the blood even up to the normal amount produces an apnoea. The following comparative experiments help to illustrate the part which the amount of air in the lungs plays during the form of apnoea. After dividing the vagi, ventilation was applied and apnoea was produced; the lungs were allowed to return to their normal volume after the ventilation and 17 secs. elapsed before the appearance of the first rhythmic respiratory movement. Both sides of the thorax were then opened and the same number of strokes of the pump applied. But at the close of ventilation the lungs were allowed to collapse completely, and the pause was found to last but nine secs. After a short time an apnoea was again produced by the same number of strokes of the pump, but the lungs were inflated above their normal volume immediately on the cessation of ventilation. The pause in this case lasted 23 secs. This experiment was repeated many times with the same result, though of course the length of the apnoea pause varied in different animals. Thus when the lungs were completely collapsed the apnoea pause lasted nine secs. whilst the fact that the lungs were inflated at the end of the ventilation served to prolong the pause to 23 secs. Thus Gad's statement that the air in the lungs under these circum-

stances is able to supply the blood with oxygen for more than one journey round the system would appear to be confirmed, and this phenomenon would seem to be one of the most important causes of the apnoea which is seen after division of the vagi.

It is interesting to notice that even during this form of apnoea the centre is sensitive to certain stimuli. If chloroform vapour is passed up one nostril during the apnoea pause consisting of a tonic inspiratory contraction, the muscles elongate at once. In fact I have found this an excellent method of estimating the amount of tone which really exists during the apnoea without having recourse to the extreme measure of dividing the phrenics. If the central end of the vagus is stimulated electrically with a stimulus just sufficient to cause an active expiration under normal conditions, exactly the same effect is produced even if the stimulus be applied during the apnoea. If the stimulus is of such a strength that it normally produces an inspiratory effect we can scarcely expect to see any change when it is applied during an apnoea pause consisting of a strong inspiratory tone. But if it is applied during the other form of apnoea, in which the muscles are almost completely elongated, a distinct rise in the curve is produced, showing that the muscles have contracted. Although the contraction does not reach the same height as if the stimulus were applied during rhythmic breathing it is sufficient to prove that the centre is still irritable, even during the form of apnoea pause, for we must not forget that this expiratory form of apnoea does not appear after division of the vagi until the vitality of the centre is probably considerably lowered.

Again, it is easy by stimulating the larynx mechanically or chemically to produce the usual movements of swallowing accompanied by elongation of the diaphragmatic muscles even though the centre is apnoeic.

To sum up the experiments detailed in this section—

(1) It is frequently possible to abolish rhythmic respiration and to produce an apnoea after the vagi are divided.

(2) The form assumed by the apnoea is in no way directly dependent on the form of the ventilation, and the rhythmic breathing during ventilation bears no relation to the rhythm of the pump.

(3) After division of the vagi ventilation with hydrogen only produces a violent dyspnoea.

(4) The forms assumed by the apnoea are very various, and the presence or absence of a tonic inspiratory contraction seems to depend on the greater or less vitality of the respiratory centre.

(5) The respiratory centre is sensitive during the apnoea to various stimuli, such as the application of chloroform vapour to the nasal mucous membrane, the mechanical or chemical stimulation of the larynx, and (to a variable extent) to electrical stimulation of the central end of the divided vagi.

(6) This apnoea is probably not caused by overarterialisation of the blood but rather by the interaction of the following factors,—

(*a*) The adaptation of the respiratory centre to a certain dyspnoeic condition of the blood in consequence of the inefficient respirations which follow division of the vagi. This would cause even normally arterialised blood to appear over oxygenated.

(*β*) The decrease in the vitality of the respiratory centre which possibly follows division of the vagi.

(*γ*) The fact that, after ventilation, when the vagi are divided, the atmosphere in the lungs is able to supply the blood with sufficient oxygen during a very considerable period.

§ 11. *On the Summation of Stimuli during Ventilation and the Nature of the Afteraction.*

We concluded above that when the vagi were intact apnoea, whether expiratory or inspiratory, was caused by the summation of certain stimuli. The question at once suggests itself where does this summation of stimuli take place? Gad[1], who was the first to offer this explanation of apnoea, believed that the stimuli were summed in the peripheral end organs of the vagi. He produced apnoea by a known number of inflations and divided the vagi during the apnoea pause, and states that the pause was distinctly shortened by this operation. I have repeated this experiment many times with all forms of apnoea but am unfortunately unable to agree with Gad that the pause is shortened by dividing the vagi; indeed in certain cases it appears to be lengthened. But these experiments are interesting from another point of view. We saw how the afteraction of the ventilation was frequently greatly hampered by the alterations in the volume of the lungs which it tended to produce. Now if the summation takes place in the respiratory centre we shall be able to observe its unhindered activity, if the vagi are divided without stimulation at the close of ventilation. The vagi remain intact during the ventilation and the summation takes place as

[1] *Apnoe*, p. 10. "Die Regulirung d. normalen Athmung," p. 28.

usual, but the moment it is complete the vagi are divided and henceforth alterations in the volume of the lungs cannot affect the activity of the centre.

No form of apnoea is so hampered by the condition of the lungs which it tends to produce as that caused by negative ventilation. We therefore find that division of the vagi at the close of ventilation considerably modifies the form assumed by this apnoea. On Curve ii, Plate VI, 20 periodic suctions produced a very inefficient apnoea. The muscles elongated quickly when ventilation ceased and rhythmic breathing began again after but a few seconds. After a short interval ventilation was again applied and the second vagus was divided at the 21st stroke of the pump. Now instead of elongating the muscles began to contract still more, and the curve rose steadily during the space of 14 secs. (Curve iv, Plate VI). Then followed a short elongation of the inspiratory muscles, which was almost at once cut short by a fresh contraction. The animal now became extremely unquiet. Now this disturbance (of which not the slightest trace had previously appeared), recalls at once the restlessness produced when the animal has breathed an atmosphere of hydrogen for some considerable time and it is probably a sign of great dyspnoea. As soon as the animal became quiet again the negative ventilation was repeated but even after 41 strokes of the pump no apnoea could be produced. Thus by dividing the vagi at the close of ventilation we have been able to convert a feeble inspiratory apnoea lasting at the most two secs. into a steady contraction of the inspiratory muscles which lasted 14 secs.

When discussing the inspiratory apnoea produced by periodic inflations when one lung had been allowed to collapse completely, I pointed out that in this case the inspiratory afteraction was comparatively little hampered by alterations in the volume of the lungs. But here again we find that division of the vagi at the close of ventilation almost doubles the duration of the apnoea and converts an almost horizontal curve into a slowly increasing contraction of the inspiratory muscles (cf. Curves v and vi, Plate VI).

I pointed out above that the apnoea produced by positive ventilation varied between two types. Curve iv, Plate VIII, is a good instance of an apnoea pause accompanied by complete elongation of the inspiratory muscles. The left vagus was divided and ventilation consisting of 15 inflations sufficed to produce an apnoea pause lasting six seconds. This pause was finally broken by a minute inspiration and 10 seconds elapsed before rhythmic breathing was completely re-

established. After a short interval the drum was again set in motion and ventilation was begun. At the twelfth inflation the right vagus was divided. A pause followed which lasted 4½ secs. (Curve iii, Plate VIII). The inspiratory muscles then began to contract just as in the previous case. But both vagi are divided and expansion of the lungs no longer checked the inspiratory activity of the centre. The muscles therefore did not relax but continued to contract steadily for 14½ secs. This strong inspiratory contraction was followed by a complete elongation, and the breathing settled down into the type so often seen after division of the vagi. Ventilation was now begun again but after 25 strokes of the pump rhythmic breathing still continued, although in a diminished form. Now, in this case at any rate, the afteraction still affected the respiratory centre even after the vagi were divided. For considering that twice as many strokes of the pump failed to produce an apnoea pause after the vagi were divided it is very unlikely that the pause which followed the division of the vagi at the close of ventilation was due to the increased oxydation of the blood. As we have already seen division of the vagi in the normal animal produces a strong inspiratory contraction, and it is interesting to notice how the last remains of the afteraction of the ventilation modify this inspiration. Normally division of the vagi is followed by the appearance of a quick strong inspiration lasting four or five seconds. But when the vagi are divided at the close of positive ventilation, the contraction which follows the apnoea pause is slow and gradual and lasts 14 secs. Thus the fact that positive ventilation has preceded the division of the vagi appears to increase the duration of the first inspiration four or five times. The slowness of the contraction is probably due to the remains of the afteraction which still tends somewhat to depress the inspiratory activity of the centre; for the afteraction is probably still exerting some influence on the centre even though the apnoea pause is broken by an inspiratory contraction.

Exactly the same phenomenon appears with great clearness in those cases in which positive ventilation produces a slow contraction of the inspiratory muscles in place of a pause. Curve i, Plate VIII, is a good instance of this type of apnoea. As soon as the ventilation ceases the inspiratory muscles begin to contract slowly. Ten seconds after the close of ventilation the first expiratory elongation appears and rhythmic breathing is slowly regained by an increase in the strength, but decrease in the duration, of the inspirations. After a pause, positive ventilation is again started and the left vagus is frozen at the 19th

stroke of the pump. Ventilation is stopped after 26 inflations and the right vagus is at once placed on the freezing apparatus (Curve ii, Plate VIII). The apnoea pause begins just as before though the muscles contract slightly less rapidly. However, 10 seconds after the cessation of ventilation they have apparently reached almost exactly the same amount of contraction as in the normal animal. But instead of elongating they continue to contract; for the change in volume of the lungs is no longer able to affect the centre. The curve therefore rises to a point far above the crests of the normal inspirations, and the first expiratory elongation does not appear until 25 seconds after the cessation of ventilation. We now see the effect that would be produced by the slow contraction of the inspiratory muscles which sometimes follows positive ventilation, provided it were not hampered by the expansion of the lungs. The first part of the curve is almost exactly the same in both cases, but whereas the contraction is checked in the normal animal after 10 secs., it continues to increase for more than twice this time if the vagi are divided. Here again the fact that periodic inflations of the lungs preceded division of the vagi seems to enormously increase the inspiratory activity of the centre.

It is sometimes possible to produce an apnoea pause after the vagi have been divided with the same number of strokes of the pump as were required to produce apnoea in the normal animal. But in such a case, apnoea before and after division of the vagi were very different in character. After division the apnoea pause was characterised by a moderately strong inspiratory tone which was considerably weaker than that produced by negative ventilation and was very different from the complete relaxation of the inspiratory muscles which followed positive ventilation. Now in such a case as this, if the periodic stimuli undergo summation in the end organs of the vagi, it is difficult to understand why the breathing does not assume the form of a blood apnoea immediately the vagi are divided. That is to say, we should expect that, when the vagi were divided during the apnoea, the inspiratory muscles would at once contract to the same extent as during the blood apnoea. But this is by no means the case, and in the experiment in question the muscles remained in a condition of complete elongation even longer than normal though the vagi had been divided at the close of ventilation.

When the vagi are divided at the close of compound ventilation the apnoea is very markedly prolonged. On Curve i, Plate VII, compound ventilation produced but an insignificant pause lasting but four secs. I

have purposely chosen this experiment for it very distinctly shows the way in which the expansion of the lungs tends to influence even the apnoea of compound ventilation. The same ventilation was now repeated and one vagus was divided at about the 14th stroke of the pump, the other at the close of ventilation. The muscles now remained in steady tonic contraction for 10 seconds (Curve ii, Plate VII). The rhythmic breathing was then regained by an increase in the strength both of the inspirations and expirations. Thus the division of the vagi at the close of ventilation, far from cutting short the apnoea, converted a short and feeble pause into a steady apnoea of twice the length. In this case again the same number of strokes of the pump were unable to cause apnoea after the vagi were divided.

The experiments in the section would seem to show

(1) That whatever the form assumed by the ventilation the periodic stimuli undergo summation in the respiratory centre and not in the peripheral end organs of the vagi.

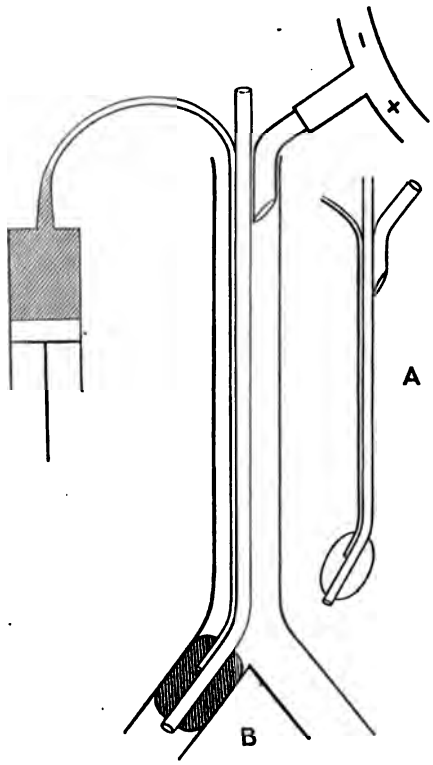
(2) If the vagi are divided at the close of ventilation it is possible to watch the effect produced by the afteraction when unhampered by afferent impulses from the lungs.

§ 12. *On Adaptation and the Phenomena which accompany it.*

It is extremely important to know what effect is produced on the breathing when one or both lungs remain permanently at the same volume, whether above or below the normal. But when we were discussing the phenomena which follow inflation or collapse of the lungs, I pointed out that the results were always complicated by dyspnoea. If for instance the lungs are inflated the trachea must remain closed during the whole duration of the inflation and the air in the lungs must stagnate. Moreover the stimulation of the vagi caused by the inflation produces a cessation of rhythmic breathing lasting some 20 secs. This again helps to prevent the renewal of the air in the lungs. During all this time the blood is becoming increasingly dyspnoeic and it is less and less possible for the centre to react in a normal manner. Thus if we wish to examine the changes in the activity of the respiratory centre produced by allowing the lungs to remain permanently at a certain volume it is necessary to find some means of eliminating the dyspnoea.

I have succeeded in overcoming this difficulty in the following manner. A cannula was constructed on the principle of Marey's Cardiac sounds. A fine tube (see figure *A*) was so bent that it could be

passed with ease down the trachea into the right bronchus. To this tube was soldered a still finer, tube which was slightly shorter and opened freely just below the bend. A piece of thin indiarubber tubing was



passed over the far end of both tubes and tied firmly at the bend and at the extreme end of the larger tube. The smaller tube, which is shorter than its fellow, now opens into a chamber formed by the indiarubber tubing, and fluid forced down the smaller tube will distend the walls of this chamber. The larger tube on the other hand passes right through the chamber and opens freely beyond it. If this cannula is passed down the trachea (see figure *B*) into the right bronchus distension of the indiarubber chamber will shut off the right lung entirely except for the opening afforded by the larger tube of the cannula. But advent of air to the left lung will not in any way be hindered, for it is easy to arrange the size of the cannula so that when it is in position at least half of the trachea remains perfectly free. A piece of brass tubing, with a wide bore, is attached to the cannula at such a height that when

the indiarubber bag lies in the right bronchus the divided trachea just slips over the end of the tube. A thread, passed round the trachea below the opening, is then tied firmly round the whole apparatus just as if an ordinary tracheal cannula had been inserted into the windpipe. By this means the trachea is kept open and the cannula remains in its proper position. It is now possible to apply ventilation to the left lung, without affecting the right lung, by connecting the mouth of this wide brass tube with the tubes from the pump. I have found it best to inflate the indiarubber chamber with glycerine by means of a stiff syringe, which is connected with the smallest tube of the cannula by means of a piece of nondistensible but elastic catheter tubing.

The right side of the thorax is opened and the lung allowed to collapse completely. The effect of the collapse gradually wears off and the animal breathes rhythmically though it is somewhat dyspnoeic. If the left vagus is now divided and each vagus only supplies the lung of its own side an alteration in the volume of the left lung will no longer directly affect the action of the respiratory centre. If the cannula is now inserted into the right bronchus and the indiarubber chamber be inflated, we are able to distend the right lung without affecting the volume of the left lung. Moreover the volume of the right lung remains unchanged no matter what respiratory movements the animal may make, for the right half of the thorax is freely open. But although the left lung is perfectly open to the air inflation of the right lung produces such a long standstill in the breathing that the animal of necessity becomes dyspnoeic. Ventilation is therefore kept up continuously on the left side all through the experiment whether the animal is breathing rhythmically or not. Thus one lung remains at a constant volume whilst the air in the other is kept of a constant composition. If the vagi do not cross this ventilation does not in any way directly affect the activity of the respiratory centre, for the left vagus (supplying the lung which is periodically inflated) has been divided before the experiment began. Now we might suppose that, even if the vagi did not cross, ventilation would still produce an apnoea such as is seen after division of the vagi. However, as far as my experience goes, this is never the case. It may be that ventilation of one lung only is not sufficient to produce an apnoea; or perhaps the fact that one vagus is still intact prevents the vitality of the centre sinking to a point sufficient for the production of a blood apnoea. Whatever the cause may be, the breathing remains steadily rhythmic during the whole duration of the ventilation of the left lung, provided the vagi do not cross at all. Now

it is extremely important for these experiments to know whether the vagi cross or not. Luckily we possess a very simple test, for if the vagi cross sufficiently to affect the breathing, closure of the trachea in the expiratory phase, after the cannula is in situ, must produce the usual strong inspiratory changes. The amount of the change will be dependent on the extent to which the vagus of the right side supplies the left lung. Again, if the vagi cross, even slightly, the breathing gradually takes up the rhythm of the strokes of the pump, and if the crossing is considerable an apnoea gradually appears. Any animal in which these phenomena appear is therefore useless for the experiments I shall now describe.

Now after we have assured ourselves that the vagi do not cross, compound ventilation is started on the left side of the thorax and the animal breathes rhythmically without any signs of dyspnoea although the right lung is collapsed. The right lung is now inflated through the cannula which has been passed into the right bronchus. All rhythmic breathing at once ceases as usual. But the ventilation now keeps the air in the left lung in exactly the same condition as if the animal was breathing rhythmically and the blood no longer becomes dyspnoeic. The pause in the breathing may be broken after a certain interval by rhythmic inspirations, but I shall first describe a case where this did not occur not because it is the rule but because, when rhythmic movements appear, they are simply superposed on the phenomena now to be described. After the pause has lasted 10—15 secs. the lever begins to rise very slowly in consequence of a slow tonic contraction of the diaphragm (cf. Curve v, Plate VII). The contraction increases very gradually, and it finally remains stationary, the lever tracing a horizontal line at a variable height above the base line. If the right lung still remains at the same volume extremely small oscillations appear on the curve, which gradually increase in strength (Curve v, Plate VIII). This is the beginning of rhythmic respiration. The muscles elongate and contract with ever increasing strength until regular rhythmic breathing is reestablished in spite of the fact that the right lung is still considerably above the normal volume.

The results which follow the inflation of the collapsed lung generally differ somewhat from this type; for the pause is mostly broken sooner or later by an inspiration (cf. Curve vi, Plate IX). This inspiratory contraction is strong and quick and traces a sharp crested curve. It is followed by a second pause, which is broken by a second inspiration. But it is a curious fact that this inspiration is smaller than its

predecessor. Each pause is now shorter and each inspiration smaller than the one which preceded it. But the diaphragm has now begun to fall into the condition of slow tonic contraction which I have just described. The ever decreasing rhythmic inspirations are therefore superposed on a steadily increasing tonic contraction of the inspiratory muscles. Rhythmic breathing then disappears entirely and the rest of the curve, including the reestablishment of the rhythmic breathing, is exactly similar to the type I described above in which the pause was not interrupted at all by rhythmic inspirations.

Thus it appears that if the right lung is kept permanently above the normal volume, the centre gradually adapts itself to the altered volume in a very peculiar manner and rhythmic breathing begins again.

If we wait until the animal breathes regularly in spite of the distension of the right lung and then allow the lung to return to the volume which it normally occupied or to collapse completely, a strong inspiratory contraction appears. The muscles contract quickly and the rhythmic breathing disappears or becomes very small. After a few seconds the rhythmic breathing becomes stronger but a considerable amount of tonic contraction still remains. Finally, after a certain length of time all tone has disappeared and the breathing presents all the characteristics of normal rhythmic respiration although the right lung is now either collapsed or much diminished in volume.

If the right lung is allowed to collapse completely when the tonic contraction which appears during the inflation is at its height, this tonic contraction is still further increased (Curve vi, Plate IX). The curve rises still further, and after a considerable interval the small oscillations make their appearance, marking the advent of rhythmic respiration. We might have expected that collapse of the lungs at the height of the tonic contraction which appears during the inflation would have caused rhythmic breathing to begin at once. But as a matter of fact the collapse rather retards than hastens the appearance of rhythmic respiration.

If on the other hand the lung is allowed to collapse very slowly, at the same point in the experiment, the tonic contraction does not increase but the lever continues to trace a horizontal line (Curve v, Plate VII). The muscles remain contracted to the same extent for about 10 secs. after the right lung had completely collapsed and rhythmic breathing then began in the usual manner. Thus here again we see the difference between a sudden collapse of the lungs and a very gradual return to the

normal volume. The former produces a strong inspiratory effect, whilst the latter produces scarcely any change in the respiratory curve.

These experiments are obviously open to many sources of error. After all the technical preparations for the experiment have been successfully made we still have to assure ourselves that the right vagus does not supply the left lung. If the breathing shows a tendency to take up the rhythm of the ventilation or if closure of the trachea in the expiratory phase produces the slightest effect on the breathing after the cannula is in situ, the animal must be rejected as unfit for these experiments. It is therefore impossible to repeat such experiments as these with the ease and certainty with which we can produce the other phenomena described in this paper.

Even when everything has succeeded we have to make quite sure that the lung has not collapsed slowly during the long inflation. The opening in the right side of the thorax should therefore be made large enough to leave the expanded lung room and to allow the experimenter to see at a glance if it still remains at the original volume. But this control is very rough. However, do not I think it likely that this error can have crept into the experiments I have just cited for the following reason. In all cases a strong inspiratory effect was produced by opening the tube in connection with the inflated right lung. Now if the lung had gradually collapsed during the duration of the inflation, it is difficult to see how opening a free passage to the lung could produce any effect.

Again, the ventilation must not be too strong. For if the periodic alterations in the volume of the left lung are too great or if the opening on the right side of the thorax be too small, every inflation of the left lung causes it to press on the inflated right lung. Thus at every stroke of the pump the right lung is diminished in volume owing to the pressure of the expanded left lung, and the breathing gradually becomes synchronous with the ventilation. This source of error may be avoided by making a wide opening on the right side of the thorax, by carefully regulating the ventilation and by not inflating the right lung too violently.

These experiments would seem to show—

(1) That if the animal is breathing rhythmically the first effect of a permanent increase or decrease in the volume of the lungs is to cause the abolition of rhythmic breathing. If the lungs are inflated the inspiratory muscles remain in a condition of complete elongation; if on the other hand they are diminished in volume the inspiratory muscles fall into a condition of tonic contraction.

(2) But in either case, provided the advent of dyspnoea is averted, the centre ultimately adapts itself to the permanently increased or diminished volume of the lungs and the breathing again becomes rhythmic. In the case of a permanent inflation this redevelopment of rhythmic breathing is preceded by a peculiar slow tonic contraction of the diaphragm.

(3) As soon as this adaptation has taken place the "normal" volume or zero is found to be altered. For instance, let us assume that the centre has become adapted to a certain volume *A*. The lung is then inflated to a volume *B*. We now find that as soon as the centre has become adapted to the volume *B* collapse to the volume *A* which previously produced no effect upon the centre acts as a strong inspiratory stimulus.

EXPLANATION OF THE PLATES.

Plate I.

Curve I. The uppermost line of the curve is traced by a control lever attached to the chest wall of the rabbit as described on p. 4.

The middle curve is traced by the lever of a Marey's tambour. This tambour is in connection with a large flask into the sides of which a cannula is fixed, which is tied directly into the trachea of the rabbit.

The lowest curve is traced by the moments of the anterior slips of the diaphragm separated and prepared according to the description on p. 4.

At the point marked with a cross the left vagus was placed upon the freezing apparatus. This curve illustrates the effect produced by dividing one vagus in a narcotised but otherwise normal animal.

Curve II. Follows on Curve I after the interval of 30 sec.

It shows the effect produced by freezing the second vagus in the same animal. During the first few respirations after division of both nerves the expiratory elongations are incomplete.

Curve III. Follows on Curve II after an interval of one minute.

The left phrenic nerve was divided in the neck at the point marked on the curve by a cross. The left side of the diaphragm and with it the left muscular slip is immediately paralysed and the whole level of the curve sinks. The curve traced by the level attached to the chest wall remains unaltered.

Curve IV. Follows on Curve III after the interval of 30 secs.

At the point on the curve marked with a cross the right phrenic nerve

was divided in the neck and the lowest curve traced by the lever in connection with the anterior slips of the diaphragm becomes practically a straight line. It now corresponds exactly with the curve traced by the control lever attached to the chest wall.

Curve V. Taken from a rabbit, weight 1550 grm. narcotised with $1\frac{1}{4}$ grm. Chloral Hydrate (Hypodermic Injection).

The medulla oblongata was separated from the brain above the striae acousticae and the left vagus had been divided. At the point on the curve marked with a cross the right vagus was frozen. The curve shows the immense inspiratory contractions thus produced.

Curve VI. From a rabbit weight 2000 grm. narcotised with $1\frac{1}{2}$ grm. of Chloral Hydrate (Hypodermic Injection).

The middle curve is traced by the writing point of a mercury manometer in connection with the carotid.

Both vagi were frozen and at once removed from the freezing apparatus. An inflation of the lungs was now found to produce no change in the breathing owing to complete interruption of conducting power in the vagi.

After an interval of 10 min. an inflation was found to produce the peculiar inspiratory effect seen on this curve.

Curve VII. The right phrenic nerve was dissected out in the neck and stimulated electrically.

The first stimulation began at the height of an inspiratory contraction and ended just before a normal inspiration had reached its full strength. The difference between the rate of contraction and elongation of the muscles during a normal respiratory movement and during a contraction induced by stimulation of the phrenic nerve is very evident.

The second stimulation of the phrenic began and ended in the expiratory period.

Two lines are drawn upon the curve to represent the curve traced by the up and down movements of the lever when the drum is at rest.

Curve VIII. Shows the effect produced by closing the trachea during the expiratory pause.

The trachea was suddenly closed at the point marked with a cross (+) and was opened again at the point marked with a circle (○).

Curve IX. Shows the effect produced by closing the trachea at the height of the inspiratory period.

The trachea was suddenly closed at the point marked with a cross (+) and was opened again at the point marked with a circle (○).

Curve X. Is selected to show the typical activity of the inspiratory muscular slips during respiration.

The slightly curved lines superposed upon the curve represent the curves traced by the lever when the drum is at rest.

Curve XI. The uppermost of the three curves is traced by the control lever attached to the chest wall.

The dotted middle curve shows the movements of a mercury manometer connected with the trachea. When the lungs are inflated the manometer registers the rise of pressure and serves as a time marker and also as a useful guard against too violent inflation.

The lowest curve, traced by the movements of the muscular slips, shows the effect produced by an inflation of the lungs when the expiratory period is incomplete and a certain amount of tonic inspiratory contraction is present even during rhythmic respirations. This condition was produced by admitting a small amount of air into the right side of the thorax (slight pneumothorax).

During the first three respirations on this curve the expiratory pause is absent and each inspiration begins before the inspiratory muscles have completely relaxed. The gentle inflation (at the point marked with a cross) first causes the cessation of rhythmic inspiration and then the gradual abolition of the permanent inspiratory tone, as shown by the slow fall of the lever. As soon as the lungs were allowed to collapse a strong tonic inspiration is produced lasting nearly two seconds. Even when rhythmic breathing begins again the expiratory elongations are very incomplete.

Curve XII. This curve was traced by a lever which described a vertical line when the drum was at rest.

The right vagus has been divided and the left side of the thorax opened. In those cases where each vagus is distributed only to the lung of its own side no effect is produced by closing the trachea in the various phases of respiration. The present curve was however taken from an animal where these conditions were not fulfilled and shows the peculiar result produced by closing the trachea in the expiratory phase. Not only are the rhythmic inspirations increased in strength as on Curve VIII but a certain amount of inspiratory tone appears between the inspiratory contractions.

Curve XIII. Shows the effect produced by a long-continued inflation of the lungs. The inflation began at the point marked with a cross (+) and the lungs returned to their normal volume at the point marked with a circle (○).

Plate II.

The dotted tracings on all the curves of this and subsequent plates represent the movements of a mercury manometer connected with the trachea. Thus any rise on the curve represents a rise of pressure in the lungs after closure of the trachea; on the other hand a fall in the curve shows that

suction has been applied to the orifice of the trachea in order to diminish the volume of the lungs.

Curve I. From a rabbit weighing 1350 grm. Chloral $\frac{3}{4}$ grm. At the point where the manometer curve rises (dotted curve) the lungs were inflated with pure oxygen. The animal was allowed to breathe pure oxygen for four or five inspirations preceding the inflation to insure inflation with the pure gas.

Curve II. Taken from the same animal.

The lungs were inflated with air at the point marked by the rise in the manometer curve.

Curve III. The lungs were inflated and allowed to return to their normal volume just before the time when the strong interrupting inspiration was expected to appear according to previous inflations of the same strength in the same animal.

Return to the normal volume was in this case accompanied by a great outburst of inspiratory energy. The slight fall in the level of the muscular curve is purely passive, as is shown by the coincident fall of the lever attached to the chest wall (the uppermost curve).

Curve IV. The left vagus was stimulated electrically with an interrupted current during the period marked by the rise on the excitation marker curve (the curve just above that traced by seconds pendulum). The stimulus chosen was of such a strength as, from previous experiments, was found to just fail in producing Rosenthal's "Tetanus" of the diaphragm (vide Curve XIII, which shows the effect produced by a stimulus of this strength in the same animal). During the stimulation both lungs were inflated for a period marked by the rise of the manometer curve (dotted). This inflation caused instant elongation of the inspiratory muscles. But as soon as the lungs were allowed to return to the normal volume the muscles again contracted under the influence of the electrical stimulation of the vagus and fell into a condition of complete tonic contraction. Thus an electrical stimulus which was unable to completely abolish rhythmic breathing was enabled to do so when aided by the inspiratory after effect of an inflation.

Curve V. The right vagus was divided and the left half of the thorax completely opened by excising portions of one or more ribs.

Inflation of the lungs produced the usual arrest of inspiration, but the return of the lungs after the inflation now produced an enormous tonic inspiration which only gradually gave way to rhythmic breathing.

Curve VI. From a rabbit weighing 1500 grm. Chloral $1\frac{1}{4}$ grm.

The lungs were inflated during normal respiration and allowed to return

to the normal volume before the pause so produced had been broken by an "interrupting" inspiration.

Curve VII. The medulla oblongata in this animal had been separated from the rest of the brain by an incision passing just above the striae acousticae. The animal continued to breathe rhythmically after the operation.

At the point marked with a cross (+) the lungs were slightly diminished in volume by suction and allowed to return to their normal volume at the point marked with a circle. In the case the negative afteraction (expiratory) following the inspiratory effect produced by the diminution in volume of the lungs is extremely well marked.

Curve VIII. From a rabbit weighing 1600 grm. Chloral 1 grm.

The right vagus has been divided and the curve shows the effect produced by complete collapse of the left lung caused by perforating the left half of the thoracic wall.

The left lung collapsed at the point on the curve marked with a cross, and a very strong tonic inspiratory contraction was produced which lasted nearly 25 sec.

Curve IX. From a rabbit weighing 1850 grm. Chloral $1\frac{1}{2}$ grm.

This curve shows the inspiratory effect produced by a "momentary" diminution in the volume of the lungs (suction) during normal respiration.

The manometer curve serves to register the duration of the suction.

Curve X. Is taken from the same animal as Curves I and II, with which it should be compared, and illustrates the effect produced by a "momentary" inflation by the lungs with pure hydrogen.

Curve XI. Shows the effect on normal respiration of sudden collapse of the right lung following perforation of the right half of the chest wall.

Curve XII. Both vagi have been divided. The central end of the right vagus was placed upon the electrodes and stimulated with an interrupted current of sufficient strength to produce active expirations. These active expirations appeared upon the muscle curve as small irregular passive movements, and affected both the muscle curve and the control curve (from chest wall) in the same direction and to the same amount.

This curve illustrates the strong inspiratory after effect which follows the cessation of an expiratory stimulation when both vagi are divided and the centre can act unhampered by afferent impulses from the lungs.

Curve XIII. Is taken from the same animal as Curve IV, with which it should be compared.

The right vagus was stimulated with an interrupted current just too weak to produce Rosenthal's "Tetanus."

This curve shows that the so-called "Tetanus" is in reality produced far more by decrease in the completeness of the expiratory elongation than by an increase in rate of the respiratory rhythm.

Plate III.

On this Plate the dotted curves are traced by a mercury manometer connected with the trachea. During the periodic inflations or suctions of ventilation the mercury makes corresponding oscillations, thus registering and controlling the strokes of the pump.

Positive ventilation is taken to mean periodic inflations, negative ventilation periodic suctions, and compound ventilation the periodic alternation of inflation and suction.

Curve I. From a rabbit weighing 1500 grm. Chloral 1¼ grm.

This curve shows the gradual abolition of rhythmic respiration under the influence of positive ventilation. An apnoea pause is produced which would have traced a curve resembling Curve III, had not the volume of the lungs been diminished (by suction) at the point marked by the fall in the dotted curve.

Curve II. From a rabbit weighing 1850 grm. Chloral 1½ grm.

Positive ventilation caused an apnoea pause consisting of a slow contraction of the muscles of the diaphragm (as in Curve III). This rise was cut short and replaced by an expiratory pause by inflating the lungs during the apnoea.

Curve III. From the same animal as Curve I.

This curve illustrates the gradual abolition of rhythmic breathing under the influence of positive ventilation (periodic inflations). As soon as the ventilation ceased the muscular slips began slowly to contract and produced a contraction curve lasting 12 sec. A similar slow contraction was increased by a diminution in the volume of the lungs on Curve I, and cut short by an inflation on Curve II.

Curve IV. From a rabbit weighing 1650 grm. Chloral 1 grm.

This curve shows the effect by negative ventilation (periodic suctions) during normal respiration. The greater number of small oscillations on the muscular curve during the ventilation are passive. This is shown by the synchronous and similar movements on the uppermost (control) curve traced by a lever attached to the chest wall.

Curve V. From the same animal as Curves I and III.

This curve shows the peculiar alteration in the respiratory rhythm which follows the cessation of positive ventilation (of short duration, 8 strokes only) in an animal in which the apnoea pause is represented by a slow inspiratory contraction (vide Curve III).

Curve VI. From the same animal as Curve IV.

Shows the effect of 7 suctions (negative ventilation) on the normal breathing.

PLATE IV.

Curve I. From a rabbit weighing 1850 grm. Chloral 1½ grm.

Shows the effect produced by inflating the lungs during the apnoea caused by negative ventilation. The duration of the inflation is shown by the rise on the dotted curve.

Curve II. From a rabbit weighing 1350 grm. Chloral ¾ grm.

Positive ventilation (16 strokes of the pump) is sufficient to abolish rhythmic breathing but insufficient to produce a pause after the ventilation ceases.

Air is the medium of inflation.

Curve III. From the same animal. To be compared with Curve II. 16 strokes of the pump are unable to produce an apnoea pause after the ventilation ceases even though pure oxygen is used as the medium of inflation. This shows that the abolition of rhythmic respiration by positive ventilation and the production of an apnoea pause is not due to the over oxydation of the blood provided the vagi are intact.

Curve IV. From the same animal as Curve I.

During the apnoea produced by negative ventilation the volume of the lungs was diminished (by suction), thus causing a considerable increase in the inspiratory contraction which characterises this form of apnoea.

Curve V. From a rabbit weighing 2450 grm. Chloral 1½ grm.

Shows the effect produced on the normal breathing by compound ventilation (alternate inflations and suctions).

Curve VI. From the same animal as Curve V., with which it is to be compared.

Shows the alteration in the effect of compound ventilation by the substitution of pure hydrogen for air as the medium of inflation.

PLATE V.

Curve I. From a rabbit weighing 1500 grm. Chloral 1 grm.

Shows the complete abolition of the respiratory rhythm produced by positive ventilation with pure hydrogen.

Curve II. From the same animal. To be compared with Curve I.

Shows the effect of the same number of strokes of the pump when air is the medium of inflation.

A comparison of the two curves shows that the only difference between the two curves lies in the shortness of the apnoea pause which follows ventilation with hydrogen in comparison with that produced when air is used. In both cases rhythmic breathing is completely abolished.

Curve III. From a rabbit weighing 2450 grm. Chloral 1½ grm.

To be compared with Curve V.

The left vagus was already divided and the curve shows the effect produced by freezing the right vagus during positive ventilation with hydrogen. The nerve is frozen at the point marked with a cross (+).

Curve IV. From a rabbit weighing 1600 grm. Chloral 1 grm.

This curve shows the peculiar form sometimes assumed by the apnoea caused by positive ventilation.

Rhythmic breathing is abolished but the inspiratory muscles do not elongate completely but remain slightly contracted. The contraction is so slight that it might fall within the errors incident to the method if its presence were not confirmed by the sudden fall which follows stimulation of the nasal mucous membrane with chloroform vapour during this form of apnoea.

Curve V. From the same animal as Curve III.

Shows the effect produced by continuing positive ventilation with hydrogen after rhythmic breathing has been abolished. The inspirations begin again during the ventilation owing to the increasing dyspnoea.

Curve VI. From a rabbit weighing 1750 grm. Chloral 1 grm.

Shows the effects of positive ventilation (5 inflations) with pure hydrogen.

Curve VII. From the same animal as Curve VI and to be compared with it, to show the effect produced by positive ventilation (6 strokes) when air is the medium of inflation. The difference between the two curves lies, not in the ease with which rhythmic breathing is abolished, but in the length of the apnoea pause. When air is used (Curve VII) a pause results lasting 9 sec., which is broken by gradually increasing inspiratory contractions. Where hydrogen is used (Curve VI) these small contractions begin directly the ventilation ceases in consequence of the dyspnoea.

Curve VIII. From the same animal as Curve IV. to show the effect produced by positive ventilation with pure oxygen.

A comparison of Curves IV and VIII shows that the only difference between the effect produced by positive ventilation with air or with oxygen lies in the length of the apnoea pause and not in its character or in the ease with which rhythmic respiration is abolished.

PLATE VI.

Curve I. From an animal weighing 1850 grm. Chloral 1½ grm.
Shows the apnoea produced by negative ventilation.

Curve II. From a rabbit weighing 1250 grm. Chloral ½ grm.
The left vagus has been divided and the curve shows the effect produced under these conditions by negative ventilation (20 suctions). To be compared with Curve IV from the same animal.

Curve III. From a rabbit weighing 1500 grm. Chloral 1½ grm.
Shows the effect produced by compound ventilation.

Curve IV. From the same animal as Curve II, on which it follows after an interval of about two minutes.

Negative ventilation was begun and at the 22nd stroke of the pump the right vagus was frozen.

On Curve II rhythmic breathing began almost immediately upon the cessation of ventilation, whereas on this curve the muscles begin to contract slowly to form a tonic inspiratory contraction of great duration (nearly 15 sec.) as soon as the remaining vagus is divided. Then follows a period of great irregularity in which the whole animal moved violently just as in those cases where great dyspnoea was known to exist (after breathing pure hydrogen).

Curve V. From a rabbit weighing 1500 grm. Chloral ¾ grm. Slight pneumothorax existed on the right side of the thorax.

Positive ventilation lasting half a minute caused an apnoea closely resembling that usually produced by negative ventilation, for the return of the lungs after each inflation now became of more value than the effect of the inflation owing to the collapse of the right lung. The difference between this form of apnoea and that caused by negative ventilation in a normal animal lies in the form assumed by the apnoea pause, for instead of consisting of a steady relaxation of the inspiratory muscles the apnoea is here a direct continuation of the apnoea of ventilation.

Curve VI follows almost immediately on Curve V.

Positive ventilation was again begun and the left vagus was frozen after it had lasted 18 sec. When it had lasted 31 sec. the right vagus was also frozen and a lung tonic inspiration lasting over 9 sec. was produced.

The slight difference between the forms assumed by the apnoea on Curves V and VI compared with the great difference on Curves II and IV is noteworthy as showing how much the apnoea produced by negative ventilation is hampered by the dilatation of the lungs which it causes.

Plate VII.

Curve I. From a rabbit weighing 1800 grm. Chloral $1\frac{1}{3}$ grm.

Shows the effect produced by 22 strokes of compound ventilation.

Curve II follows closely on Curve I.

During the duration of compound ventilation the left vagus was frozen. At the 22nd stroke of the pump the right vagus was frozen. On Curve I, 22 strokes of the pump only produced an apnoea pause lasting about 4 sec., whereas on the curve the pause lasts over 10 sec. owing to the division of the vagi at the close of ventilation. Thus we see that, firstly, the stimuli to the end organs of the vagi undergo summation in the centre and not in the peripheral end organs of these nerves, and, secondly, that this form of apnoea is considerably hampered in the normal animal by the dilatation of the lungs which it produces.

Curve III. From a rabbit weighing 1500 grm. Chloral $1\frac{1}{4}$ grm.

Shows the effect of diminishing the volume of the lungs during the apnoea pause caused by positive ventilation. The diminution in volume of the lungs (suction) appears on the manometer curve (dotted) as a fall two seconds after the cessation of the ventilation.

Curve IV. From a rabbit weighing 1850 grm. Chloral $1\frac{1}{2}$ grm. This curve is the converse of Curve III, for it shows the effect produced by a short inflation of the lungs during the apnoea caused by negative ventilation.

Curve V. Compound ventilation was continued during the whole duration of this curve on the right side.

For a full description vide p. 57.

The right lung collapsed slowly during the period between the two circles.

Plate VIII.

Curve I. From a rabbit weighing 1500 grm. Chloral $1\frac{1}{4}$ grm.

Shows the effect produced by 21 inflations (positive ventilation).

Curve II follows closely on Curve I, with which it is to be compared. Positive ventilation is repeated in the same animal after a short interval, and the left vagus is frozen during the ventilation. After 26 strokes of the pump the ventilation is stopped and the right vagus is frozen as quickly as possible. Nevertheless the apnoea pause takes an almost absolutely identical form to that seen in the normal animal (Curve I) except that it gradually passes over into a strong tonic inspiratory contraction. Whereas in the normal animal rhythmic breathing was ushered in by an expiratory elongation after the pause had lasted 10 seconds, the first expiration does not appear for 25 seconds when the vagi were divided at the close of ventilation, showing that this

operation far from cutting short a vagus apnoea rather tends to prolong its duration.

Curves III and IV are both taken from an animal weighing 2000 grm. Chloral 1 grm.

The left vagus was divided before the experiment began.

On Curve IV an apnoea was produced by 15 inflations. After a short pause positive ventilation was begun again and the right vagus was frozen at the twelfth inflation. Yet in spite of the fact that both vagi were divided a complete inhibitory apnoea pause was produced which gradually gave place to a slow tonic contraction of the inspiratory muscles. Now it is interesting to note that on Curve IV rhythmic respiration began again after the apnoea pause by a small inspiratory contraction, which was cut short after reaching but a very small height, whereas on Curve III the pause is broken by a long slow steady rise owing to the fact that dilatation of the lungs can no longer reflexly inhibit the inspiratory activity of the respiratory centre. Thus here again we see the controlling effect produced by the stimulation of the vagi produced by a change in the volume of the lungs.

Curve V. Compound ventilation was kept upon the left side of the thorax during the whole duration of this curve.

For a full description of this curve vide p. 56.

PLATE IX.

Curve I. From a rabbit weighing 1300 grm. Chloral 1 grm.

Both vagi have been divided just previously to this curve.

Shows the tonic inspiratory type of blood apnoea which follows positive ventilation after the division of both vagi.

Curve II. From a rabbit weighing 1800 grm. Chloral 1½ grm.

Both vagi have been divided some time before this curve was taken.

Shows the expiratory type of blood apnoea which follows positive ventilation, especially when the animal is in any way exhausted or if the vagi have been divided some time before ventilation is resorted to.

Curve III shows the effect of inflating the lungs of a normal animal with chloroform vapour.

The inflation is marked by the rise on the manometer curve (dotted).

During the first few seconds following the inflation distinct traces of the normal inhibiting effect of an inflation are seen, but the breathing rapidly assumes the type seen on dividing both vagi. This tends to show that chloroform or ether vapour rather tends to paralyze than to stimulate the end organs of the vagi in the lungs.

Curves IV and V are taken from the same animal at intervals of a few minutes.

Curve IV shows the normal effect produced by inflating the lungs with air after division of the right vagus.

On Curve V the lungs were inflated in exactly the same way and apparently as nearly as possible to the same extent (to judge by the rise of the mercury manometer). But during the inflation the left vagus was frozen and we here find that the interrupting inspiration is replaced by a very strong inspiratory contraction of quite abnormal duration.

Curve VI. Compound ventilation was continued during the whole duration of this curve on the left side of the thorax.

The right lung collapsed suddenly at the point marked with a circle.

For a full description of this curve vide p. 57.

Part II. THEORETICAL, follows in a succeeding Number.

ON THE ORIGIN OF UROHAEMATOPORPHYRIN AND OF NORMAL AND PATHOLOGICAL UROBILIN IN THE ORGANISM. By C. A. MAC MUNN, M.A., M.D.

(With Five Charts of Spectra. Plates X. to XIV.)

THE following paper contains an account of a series of experiments made in order to determine the connexion between the colouring matters of blood, bile and urine.

In 1880[1] I published a paper in which I endeavoured to trace this connexion, but at that time I was unaware of the importance of the feeble bands in the red half of the spectra of solutions of the various colouring matters, which appear under the influence of such reagents as ammonia, sodium hydroxide, zinc chloride and ammonia, and zinc chloride alone. It is only by a careful study of these feeble bands that we can distinguish these colouring matters from each other and compare them with the artificially prepared pigments.

It is impossible in some cases to separate (for example) a haematin derivative from a bile derivative and for the present I have been limited mainly to a description of spectra. In time however I hope to be able to say more about their chemical characters. So far the results obtained have been so important that I feel justified in publishing them in their present state; but I regret that I have to give them in greater detail than at first sight may appear necessary. To enable anyone to follow these results, such detail is absolutely required, as will appear by and bye.

Owing to the ease and rapidity with which bodies that yield banded absorption spectra can be detected, this method of analysis applied to urine is likely to prove useful to the physician, and he has in the pigments an index of the metabolic activity which is taking place in the organism under normal and pathological conditions; besides it can hardly be doubted that the various constituents associated with the pigments must arise under like conditions with them.

Before giving my results it may not be thought unnecessary to

[1] "Further researches into the colouring matters of human urine" &c. *Pro. Roy. Soc.* No. 208, 1880.

recapitulate what is known of these colouring matters, especially as my results do not always agree with the received ideas, although they do confirm my former results so far as I have gone.

The earlier observations of Jaffé[1] and Maly[2] are known to readers of modern text-books of physiology; the former found urobilin first in the bile, then in urine, and described its green fluorescence with zinc chloride and ammonia, he also described its chromogen in urine. Maly then showed how to prepare a like substance, which he considered identical with Jaffé's urobilin, from bilirubin, and he named this hydrobilirubin, and subsequently said it was identical with the stercobilin of Vaulair and Masius[3]. He supposed that the bilirubin of the bile is changed into urobilin by reduction in the intestine, and is then absorbed in part and appears in the urine. Hoppe-Seyler[4] showed that a body with the reactions of urobilin could be obtained by the action of tin and hydrochloric acid on haemoglobin and haematin, and he could not find it in blood-serum. He says that normal urine contains no traces of urobilin, on the contrary a chromogen which gradually forms urobilin by a spontaneously occurring oxidation, an observation first made by Jaffé. Hoppe-Seyler, in his book on Physiological and Pathological Chemical Analysis, refers to Baumstark's[5] urorubrohaematin and urofuscohaematin, and says that both of these pigments stand in near relationship to haematin. Among later contributions to this subject I may mention those of Esoff[6] and Disqué[7]. The latter extended the knowledge of urobilin and hydrobilirubin considerably and showed that the hydrobilirubin of Maly cannot be considered a pure body, a statement with which I agree. He could only find the band of Jaffé's urobilin in urine after long exposure to the air, but he evidently considered hydrobilirubin and urobilin identical substances, and he found the latter most abundantly in all diseases where the urine is diminished in quantity, also in copious perspiration, in heart disease and lung diseases, especially pneumonia. I shall refer to Disqué's results further on.

[1] *Centralb. f. d. med. Wiss.*, 1863, S. 241. *Archiv f. path. Anat.* Bd. 47, S. 405, *Zeit. f. anal. Chem.*, Bd. 9, S. 105 and Bd. 3, S. 245.

[2] *Ann. Chem. Pharm.* Bd. 161, S. 368 and Bd. 163, S. 77.

[3] *Centralb. f. d. med. Wiss.* 1871, No. 24.

[4] *Archiv f. d. ges. Physiol.* Bd. 10, S. 208. *Berichte d. deutsch. chem. Ges.* Bd. 7, S. 1065.

[5] *Berichte d. deutsch. chem. Gesell.* 1874, VII. S. 1170.

[6] *Archiv f. d. ges. Physiol.* XII. S. 50.

[7] *Zeitsch. f. physiol. Chemie*, Bd. II. S. 259.

In the paper quoted above I showed that there is a distinct difference between normal and (what I there called) febrile (= pathological) urobilin, as the normal pigment is lighter in colour, being brown or yellow brown, and its solutions also possess a lighter colour than those of pathological urobilin. The absorption band at F is much less shaded, has less well-defined edges, and in dilute solutions disappears with potassium—or sodium—hydroxide[1] (other differences will be referred to in this paper). I found that it appeared to be identical with choletelin and with a body which is produced by the action of peroxide of hydrogen on acid haematin, to be described further on.

I also described in that paper a pigment which I had met with in the urine of rheumatic fever and which I prepared artificially by the action of zinc and sulphuric acid, and by that of sodium amalgam, on haematin, which I accordingly named urohaematin, but which I have since named urohaematoporphyrin[2]. I came to the conclusion that if pathological urobilin is derived from bile, it represents an intermediate stage of the oxidation of bile pigment, but I could not account for its being so like hydrobilirubin. Since then however Hoppe-Seyler has shown that nascent oxygen plays a very important rôle in these supposed reduction processes, a conclusion which fully explains away all these difficulties. It was quite evident then that under this name urobilin observers had confused three distinct pigments, (1) normal urobilin, (2) pathological urobilin, and (3) urohaematoporphyrin.

I also inferred the presence of two chromogens in urine: that of normal, and that corresponding to pathological urobilin, for by no other hypothesis could I account for the results obtained. Since however there appears to be no essential difference between stercobilin and pathological urobilin there is less difficulty in accounting for this: as the chromogen of stercobilin may be present in normal urine in very small quantity. It is quite unnecessary, and probably is incorrect, to suppose that the reduction of these colouring matters to chromogens takes place in such places as the intestine, because when brought by the blood to various tissues and organs, where oxidation is going on, they could not fail to become oxidised again. It is more probable that they are reduced in the kidney and bladder by the action of those reducing substances which are known to be present in urine. If this be so, then

[1] Whereas in the case of pathological urobilin the band at F is replaced by another nearer the red.

[2] This *Journal*, Vol. vi. Nos. 1 and 2.

it is conceivable that one part of the colouring matter might become more reduced than another, so that we might have two chromogens, one that of normal, another corresponding to that of pathological urobilin, for it would appear that the artificially prepared pigment corresponding to normal is convertible by reducing agents into that corresponding to pathological urobilin. That this occurs, at least at times, is probable from a recent observation of Salkowski[1], who found that a sample of urine peculiarly rich in urobilin seemed to lose it on keeping, and I have noticed the band disappear from urine after it had stood some time, long before ammoniacal fermentation had time to take place. But I shall refer to the subject further on.

The occurrence of urohaematoporphyrin in urine has recently been again studied by le Nobel of Leyden[2], who, while differing from me as to the presence of a band at F in acid solutions, has confirmed the truth of my statements. His methods of preparation and of observing the spectrum explain this discrepancy, as he used a flask provided with a small side tube much too narrow for showing feeble bands, and his reduction experiments were not carried far enough, as may be judged of from his statement that in his first experiment he did not find it necessary to provide his flask with a tube for the escape of gas "da keine Gasentwickelung statt fand," and he did not heat the solution, trusting merely to the "Sonnenwärme." He found that by the action of reducing agents on haematin there were successively formed a series of reduction products which were named by him (1) haematoporphyrin, (2) haematoporphyroidin, (3) isohaematoporphyrin (= urohaematoporphyrin), and (4) urobilinoidin. The last was not (he says) identical with urobilin, although like it, but he found that it passed back again into urohaematoporphyrin : if so the statement that the latter possesses no band at F carries its own contradiction, for no one knows of a chromogen or reduction product possessing a band at F that does not give that band intensified in its oxidised state. I need not refer to his other remarks, as one was based on a mistranslation of the word "doubtless," and the other on his ignorance of my having changed the name urohaematin to urohaematoporphyrin in this Journal[3].

[1] *Chem. Centr.* XVIII. 1089.

[2] "Ueber die Einwirkung von Reductionsmitteln auf Haematin und das Vorkommen der Reductionsproducte in pathologischem Harne." *Archiv f. d. ges. Physiol.* Bd. XL. 1887, S. 501.

[3] This *Journal*, vol. VI. Nos. 1 and 2.

It is quite evident from le Nobel's and my own observations that reduction products of haematin occur in the urine under pathological conditions, and he found such present in acute rheumatism, croupous pneumonia and cirrhosis of the liver, to which I may now add so-called idiopathic pericarditis, Addison's disease, Hodgkin's disease, measles, typhoid fever, meningitis and peritonitis. In these cases the origin of the pigment could not have been food-products, since the patients had been placed on a milk-diet (in most of my own cases), nor is it likely that the pigment had its origin in the liver.

I do not think it is an exaggeration to say that the occurrence of this colouring matter in the urine is of very great importance to the physician, indicating as it does very grave disturbance in the system. Le Nobel also found that hydrobilirubin is not identical with urobilin.

I shall describe here (1) pathological urobilin, (2) urohaematoporphyrin, (3) the artificial production of this, (4) normal urobilin, (5) its artificial preparation, (6) stercobilin, (7) hydrobilirubin and (8) the urobilin-like substance in bile[1].

The so-called urobilin from a case of tubercular peritonitis. The urine was of an orange colour. It showed a band at F, from about λ 502 to λ 479, extending in a greater depth from λ 504 to λ 475. It was treated with neutral and basic acetate of lead, the precipitate filtered off and decomposed with rectified spirit acidulated with sulphuric acid (12 parts H_2SO_4 to 68 parts spirit). The deep red filtrate showed a feeble band at D, another between D and E and a black band at F. The last had the peculiarity of shading shown by the band of pathological urobilin, of hydrobilirubin, stercobilin and of the urobilin-like substance of bile, that is, a less shaded part stretching for about its own breadth towards violet. This fluid was diluted with water and agitated in a separating funnel with chloroform, which became coloured red by the pigment and was separated off, filtered, and evaporated at the temperature of the air. The residue was a red brown amorphous pigment soluble in the urobilin solvents.

The chloroform solution was a deep red colour, and showed a band before D and one in green, besides that at F: the first about λ 619 to λ 591, second about λ 567·5 to λ 547, while the dark band nearest violet at one degree of dilution read from λ 517 to λ 464. This is a great breadth, and normal urobilin never can be made to show a band like this.

[1] It is probable that the new bands which appear on the addition of such reagents as $ZnCl_2$, NaHO, NH_4HO, H_2SO_4, HCl, and so on, belong to zinc, sodium, &c. compounds, but at present I cannot assert that such is the case.

By progressive dilution it read from λ 511 to λ 477, then λ 506 to λ 483.

The alcohol solution had a fine wine-red colour and showed a band at D, another in green and the dark band at F. The first about λ 607·5 to 587, the second about λ 577 to λ 552 (?) and that at F in a more dilute solution from about λ 504 to λ 477, with its darker part from λ 501 to λ 479 (?).

The action of ammonia on this is worthy of attention; it caused four bands to appear bearing a close resemblance to those of alkaline urohaematoporphyrin, which certainly do not appear in a similar solution of hydrobilirubin, while the urobilin band at F disappeared *in toto*; the fluid becoming lighter in colour. The narrow faint band in red, sp. 1, Chart I. read from λ 625 to λ 615, and was only seen in very deep layers, (the spectrum shown in sp. 1 being a combination of three, got by observing *three* depths of fluid,) the shading before the second band began at about λ 597 and extended to λ 581, from which number the second band became darker and extended to λ 560·5, the third reading from λ 542 to λ 521·5. The total disappearance of the band at F at once distinguishes this colouring matter from urohaematoporphyrin.

When zinc chloride was added to this ammonia-treated solution and it was filtered, it showed a splendid green fluorescence and new bands appeared: sp. 2, Chart I. in a deep layer and sp. 3 in a less deep. These read: 1st from λ 653 to λ 622, 2nd from λ 587 to λ 567·5 in its darker part, 3rd in a deep layer was of great breadth, from about λ 518·5 to λ 473, while in a shallower layer its dark part read from λ 517 to λ 494. The darker part of this band was towards the red; on its violet side it gradually shaded off; this as I formerly showed is a characteristic of pathological urobilin (as distinguished from normal). Zinc chloride alone does not produce these bands, although it does produce a green fluorescence: duller than when ammonia has been previously added. The band nearest violet also appeared double, although I have not shown this division in sp. 4, I. which represents the combination spectrum of a deep and shallow layer. The band at D read from λ 583 to λ 573, with a shading before it beginning at about λ 607·5. The darker part of the band next violet read from about λ 517 to λ 499, with a shading extending towards violet as shown in the diagram.

Sodium hydroxide added to an alcohol solution produced precipitation; after filtering, the solution was of an orange colour and showed

sp. 5, I. (a combination spectrum). The 2nd and 3rd bands shaded off into each other, and the series read as follows:—1st from λ 653 to λ 625 including shadings, dark from λ 647 to λ 627·5, 2nd λ 597 to λ 577 a mere shading; 3rd from λ 571 to λ 559 (?). The dark band read from λ 520 to λ 494, with its darkest part from λ 517—496.

Potassium hydroxide produced the same effect. Sulphuric acid made an alcohol solution redder but did not bring out the sharp narrow bands characteristic of acid urohaematoporphyrin. This solution showed a band at D from about λ 607·5 to λ 585, another from λ 562 to λ 535 and the black urobilin band from about λ 501 to λ 479 in its darker part.

Hydrochloric acid produced the same effect. Neither acid really had any appreciable effect upon the spectrum.

Here then is a typical case of the presence of pathological urobilin, and the characters given above will serve for comparison with those of the other colouring matters described below. But the action of ammonia, and of ammonia and zinc chloride, showed that the colouring matter owed its origin in part at least to haematin, as will appear by and bye.

Urohaematoporphyrin-like colouring matter from a case of supposed rheumatic origin. The person from whom the urine which contained this colouring matter was obtained had had a chill, and developed "rheumatic" symptoms followed by albuminuria; the latter disappeared and was followed by violent frontal headache with a temperature varying from 100° to 102° F. The specimen was obtained while the temperature remained high. It had a deep yellow—approaching an orange—tint. Treated with some sulphuric acid, a trace of the acid urohaematoporphyrin bands was visible. The urine was precipitated with neutral and basic lead acetate, the precipitate separated by filtering, and decomposed with acidulated alcohol ($3H_2SO_4$ to 17 rect. sp.). The filtered extract gave the acid urohaematoporphyrin bands well marked sp. 6, I.: these read as follows:—1st from λ 597 to λ 587; 2nd a shading about λ 577 to λ 567·5, 3rd from λ 557 to λ 540, and 4th from λ 504 to λ 483. (Note the feebleness of shading of band at F and compare with urobilin band.) On agitating the above solution (after dilution with water) with chloroform this assumed a yellow colour, and on evaporation it left a brown residue without a trace of red, and was slightly altered by heating on the water bath as it was not now soluble completely in chloroform, the chloroform showing the urohaematoporphyrin bands, of which that at F was darker than before. What

was insoluble in chloroform went into absolute alcohol, forming a dull brownish-yellow solution by reflected gaslight, but more of an orange tint by transmitted gaslight, and showing the bands of neutral urohaematoporphyrin.

The part of the above residue soluble in chloroform may be called (A), and that insoluble in chloroform but soluble in absolute alcohol (B).

(A) On evaporating the chloroform a brown residue was left perfectly soluble in absolute alcohol, having a reddish yellow tint by transmitted gas-light. It was worthy of notice that in this solution the band at F was darker than in the case of a similar solution of (B.) Otherwise the spectrum was that of neutral urohaematoporphyrin, but I hesitate to give the measurements of the bands as they were very faint. With ammonia however there was only one band in red as shown in sp. 7, I. which proves that this pigment had gone beyond the stage of true urohaematoporphyrin, and which also shows the close connexion between urohaematoporphyrin and pathological urobilin. The band at F did not disappear under the influence of the ammonia but was moved nearer the red end, as in the case of urohaematoporphyrin.

With zinc chloride and ammonia a green fluorescence was obtained and sp. 8: the bands of which read as follows:—1st band in its darker part from λ 585 to λ 553·5, 2nd band about λ 514 to λ 499.

(B) The alcohol solution of (B) on treatment with ammonia also gave sp. 7, I. And the band at F was not removed by the ammonia, but was shifted nearer the red end and made less distinct. On adding zinc chloride to an ammoniacal solution a spectrum similar to sp. 8, I., was obtained but the green fluorescence was not very distinct at first, but became better marked on standing. It was noticeable too that the band which is produced by zinc chloride and ammonia in the case of pathological urobilin was not in this case nearly as dark, nor as well defined.

With zinc chloride alone only an attempt at a green fluorescence was seen and two narrow bands in green appeared, as well as one before F; this was better marked on allowing the solution to stand for an hour, when sp. 9, I. was mapped. These bands read approximately:—1st λ 583 to λ 571, 2nd about λ 549 to λ 532, and 3rd about λ 517 to λ 501.

The spectra of these solutions were somewhat unsatisfactory, but on considering all the characters of this pigment and comparing them with what follows one cannot help concluding that this pigment was intermediate between urohaematoporphyrin and pathological urobilin.

It had features common to both and established the link binding them together in the clearest manner, and proving that the first specimen of pathological urobilin was in all probability derived from haematin by reduction.

Urohaematoporphyrin from a case of measles. The urine was pale, and showed a copious deposit of whitish urates. Treated with a mineral acid a faint acid urohaematoporphyrin spectrum appeared. The urine was treated precisely as before (with the lead acetates, acidulated alcohol and chloroform). The acidulated alcohol filtered extract gave the following (acid urohaematoporphyrin) bands: 1st from λ 597 to λ 587, 2nd λ 555 to λ 540, 3rd from λ 504 to λ 481. The solution had an orange colour (gaslight). On diluting with water and agitating with chloroform the latter became orange yellow (gaslight). This gave also the acid urohaematoporphyrin bands, that at F being much darker than in the acidulated alcohol extract. On evaporation a dark brown residue was left soluble in alcohol, ether, chloroform, &c.

The rectified spirit extract was brownish and gave in two different layers, sp. 10. I. Approximately these bands read:—1st λ 630 to λ 619, 2nd λ 617·5 to λ 599, 3rd λ 581 to λ 555, 4th λ 538 to λ 520, 5th λ 506 to λ 481. On adding ammonia to this a four-banded spectrum, sp. 11[1] was obtained, whose bands measured approximately: 1st λ 625 to λ 615, 2nd λ 581 to λ 562 (with a shading before it), 3rd λ 540 to λ 523, and 4th λ 511 to λ 494 (?). So that the band at F was removed nearer the red end by ammonia, whereas had it disappeared the pigment could not have been urohaematoporphyrin.

With ammonia and zinc chloride a greenish fluorescence was obtained, the solution being yellow in thin layers, and reddish in deep layers (with transmitted gaslight); and after standing some time the solution showed sp. 12, I.; the bands reading[2]: 1st λ 587 to λ 569, 2nd λ 547 to λ 529, and 3rd λ 514 to λ 499.

Zinc chloride alone produced nearly the same bands with this very important exception: that the band at F was not narrowed and did not move towards the red end of the spectrum, nor did the solution show any distinct green fluorescence. On comparing sp. 13—this spectrum—with sp. 12, this difference is well seen. The 1st and 2nd bands gave the same wave-length measurements as

[1] This should have yielded a five-banded spectrum, but the presence of one or of two bands in red seems to depend on trifling differences.

[2] A shading in red impossible to measure was present, therefore the 1st band is really the 2nd.

those of sp. 12, the third however read from λ 504 to λ 481; but after standing 24 hours it read from λ 514 to λ 499. Caustic soda added to an alcohol solution produced similar bands to those produced by ammonia, giving the following readings: 1st λ 625 to λ 615, 2nd λ 581 to λ 562, 3rd λ 542 to λ 526, and 4th λ 515·5 to λ 496.

Here then there was distinct evidence that the band at F, which le Nobel would have belonging to urobilin, really belonged to the pigment which gave the bands at the red end of the spectrum, as the action of ammonia showed; and all the bands belonged to urohaematoporphyrin. One of course cannot absolutely prove that some traces of urobilin were not present in this and in similar cases, but one can prove that the principal pigment present was urohaematoporphyrin, and that this does possess a band at F like urobilin.

Urohaematoporphyrin-like pigment, &c., from a case of meningitis. The urine was orange in colour and showed only a band at F, but on treatment with mineral acids I perceived a faint urohaematoporphyrin spectrum. The urine was as before precipitated with neutral and basic lead acetate, the precipitate after separation by filtering treated with alcohol acidulated with sulphuric acid, of the same strength as before. The acidulated alcohol extract had a deep red colour in deep, and an orange in shallow layers, by gas-light, and showed the following bands: 1st λ 597 to λ 587, 2nd λ 557 to λ 542, 3rd λ 506 to λ 475. The last band was very broad and very dark, closely resembling that of pathological urobilin, an inference which was supported by the much redder colour of the solutions than in the last case.

The chloroform solution (got by shaking the acidulated alcohol extract with chloroform) was of a fiery red colour in deep, and orange in thin layers by gaslight; in it the urohaematoporphyrin bands were faint, but an intensely black band was present at F. It began at λ 511 and extended to λ 473. This solution left on evaporation a red-brown residue, not the dull brown of urohaematoporphyrin.

The rectified spirit solution of this residue gave sp. 14, I., which represents the result of examining three different depths of solution. In spite of the fact that although the principal colouring matter here appeared to be urobilin, yet on adding ammonia the spectrum came to resemble that of alkaline urohaematoporphyrin, as sp. 15 shows. After standing some hours this fluid without any zinc chloride showed a green fluorescence. The two faint bands in red were most difficult to measure: the 1st may have been from λ 647 to λ 636, 2nd λ 625 to λ 615, the darker part of 3rd λ 583 to λ 559, 4th λ 547 to λ 526 (?) and 5th about

λ 514 to λ 499 (?). The last got much darker after the solution had stood for some hours. With zinc chloride and ammonia a fine green fluorescence appeared; some faint bands were seen in red, but the most prominent bands which alone could be mapped are shown in sp. 16, that after D read approximately from λ 585 to λ 557 in its darker part, the other from λ 512·5 to λ 501, but it was continued into a less shaded part, extending to about λ 492.

With zinc chloride alone sp. 17, I. appeared, the fluid showing a distinct green fluorescence. The bands reading: 1st λ 583 to λ 567·5 (in its darker part), 2nd about λ 549 to 532 (?), 3rd in its darker part λ 514 to λ 499, although a shading extending to λ 473 was visible. On dilution this fluid had a rose tint with a splendid emerald green fluorescence and then two bands at F were visible. The first one darker than the second from about λ 517 to λ 499, the other beginning where this one ended, and extending to about λ 483. After the lapse of 20 minutes only the first of these was visible, but after standing 24 hours the dark band of sp. 17, I. was again seen; so that the action of the zinc chloride was only transient in this case. On diluting the fluid which showed this dark band, the latter again appeared double; and another very curious appearance, which doubtless was an optical delusion due to contrast, was this: in a dilute solution the band nearer red seemed the darker, whereas in a stronger solution that nearer violet appeared the darker.

When caustic soda was added to the rectified spirit solution a feeble band appeared in the red, that after D was darkened, and that at F was at once replaced by a narrow dark band. These read approximately: 1st λ 627·5 to 597, 2nd λ 581 to λ 553·5, and 3rd 517 to λ 499 in its darker part.

The separating funnel seemed, after the chloroform had removed the above pigments, to contain a considerable amount of urohaematoporphyrin, and even a second extraction still left some present in the acidulated alcohol. And I was led to conclude that the first chloroform extract contained much pathological urobilin with some urohaematoporphyrin, the second more of the latter with the former still present, while the acidulated alcohol finally contained very little of the former, but did contain the latter. To compare the pigments removed by a second chloroform extraction with those just described, I may add here the result of an examination of solutions of the pigment obtained by this second chloroform extraction. The residue was of a reddish brown colour. Its rectified spirit extract gave practically the same spectrum as sp. 14, I., except that the feeble band in red (not figured)

was more distinct, and read about λ 627·5 to λ 616, 2nd λ 542 to λ 523 (?), the band at F being quite black, and reading from about λ 504 to λ 473 including shadings. With ammonia the bands remained much the same as those of sp. 15, I., as they now read: 1st λ625 to λ615, 2nd in its dark part λ 581 to λ 557, 3rd λ 542 to λ 523, and 4th about λ 511 to λ 494. With zinc chloride and ammonia the spectrum was almost the same as sp. 16, I., except that a band in red from λ 625 to λ 615 was visible also.

The action of zinc chloride alone, and also of sodium hydroxide, showed that in the second chloroform extract, as I have already stated, relatively more urohaematoporphyrin, relatively less pathological urobilin, was present. So that it is easy enough to determine in a given case when a mixture of pigments is present.

It would appear that zinc chloride enables one to distinguish pathological urobilin from urohaematoporphyrin and—as will afterwards be shown—from normal urobilin.

Urohaematoporphyrin from a case of typhoid fever. The urine was orange in colour; it showed some shading in the green and a badly defined band at F. With sulphuric acid, a faint shading before D and another in green were visible, also a badly marked band at F. These bands seemed to be those of acid urohaematoporphyrin. The urine was treated as before. The acidulated alcohol extract of the lead precipitate was orange-red, showing the following bands: 1st λ 599 to λ 585, 2nd about λ 555 to λ 538[1], and 3rd about λ 506 to λ 479, this being badly defined at the edges. It was noticeable that these bands were extremely faint at first and got gradually darker, showing that a chromogen (= le Nobel's urobilinoidin?) belonging to urohaematoporphyrin was present, which under the action of acid and air became converted into the latter body. On agitating with chloroform and evaporating this, a brown amorphous residue was left. This dissolved in rectified spirit, forming a yellow-red solution, showing the neutral urohaematoporphyrin spectrum; on adding ammonia sp. 19, I. appeared, the band before F not being there shown, which extended from λ 514 to λ 492, and hence belonged to urohaematoporphyrin, not to pathological urobilin. With ammonia and zinc chloride a green fluorescence was obtained, while the spectrum appeared as shown in sp. 20, I., of which the bands read: 1st λ 585 to λ 571, 2nd λ 553·5 to λ 532 and 3rd about λ 514[2] to λ 494.

[1] A shading was seen like that of Sp. 6, I. between the two first bands mentioned above.

[2] Or λ 517.

This pigment is therefore urohaematoporphyrin, and I do not think any urobilin was present with it.

Pathological urobilin from a case of intraperitoneal haemorrhage. The specimen of urine was sent to me from University College Hospital by Mr Dean and Mr J. Rose Bradford. The patient, aged 35, had been operated on, on April 19th, for the radical cure of hernia. A few hours later he became cold and collapsed. Twenty-four hours after the operation the temperature rose to 105°·8 F. And in the course of 12 hours it fell to 99°·6. No peritonitis appeared to be present. Until April 25th the urine appeared normal, but on that day it was found to contain a-sixth albumen with some tube-casts, but no blood corpuscles. Now *a priori* one would expect to find that in such a case urohaematoporphyrin ought to be present, but it was not; the pigment being evidently pathological urobilin. This specimen also contained indoxyl-sulphate of potassium, skatol (probably), and did not contain either bilirubin, haematin, haemoglobin or methaemoglobin, although its colour was orange-red. On May 3rd I received enough fluid to enable me to thoroughly examine the colouring matters. The urine was treated as before. The acidulated alcohol extract was at first a bright red colour, becoming duller in tint after standing; a deep layer showed only red and a little green, with a feeble shading before D; in a thinner layer a second in green and a black band at F was seen. The chloroform extract of this showed a shading before D, another feeble one in green and a black band at F. The latter began at about λ 514, was deeply shaded at about λ 510 and dark up to λ 479, gradually shading off to about λ 464. Now this very dark broad band was not seen in any solution of urohaematoporphyrin. The chloroform left on evaporation a reddish-brown residue, which when dissolved in rectified spirit gave sp. 1, Chart II. the band at D reading from about λ 605 to λ 573, and that at F about λ 504 to λ 477 in its darkest part, and being continued into a shading extending towards violet.

With ammonia added to this solution sp. 2, Chart II. was seen, the band there shown beginning at about λ 601 as a feeble shading and becoming darker from λ 587 to about λ 562; and the band at F had gone, leaving no trace of another in its place.

When treated with ammonia and zinc chloride this alcohol solution showed a splendid green fluorescence and sp. 3, Chart II. was seen. It was impossible to measure these bands, that in red extended to about λ 625, the second began as a feeble shading about λ 601 and got darker at λ 591, extending to about λ 566, the band before F in its darker part

read from λ 514 to λ 494 and shaded off to about λ 483 or further, towards violet.

Zinc chloride alone developed also a splendid green fluorescence, the band at F being shifted towards red, and not being double : it began to be deeply shaded at λ 515·5 and extended quite black to λ 496, and it was continued into a shading which extended to about λ 483. A very marked difference is here noticeable between this pigment and urohaematoporphyrin, as can be seen by comparing their spectra, while it showed many resemblances to "biliary" urobilin[1].

Pathological urobilin-like pigment from a case of extravasation of blood into one leg. Contrasting in many points with the last specimen was the next, sent to me by Prof. Sidney Ringer, F.R.S. Five days before the urine was obtained the patient had had blood extravasated into one leg. This specimen was also orange in colour. It was treated with sulphuric acid, but it did not then show the acid urohaematoporphyrin bands, but instead an intensely black band at F. It was treated with the lead acetates, &c., as before. The acidulated alcohol extract was a wine-red colour, showing traces of the acid urohaematoporphyrin bands, and a very black band at F. The chloroform extract of this left a reddish brown residue on evaporation. In the alcohol (rect. spt.) extract of this residue only one band, that at F, from about λ 504 to λ 479 (in its darker part) could be seen. When ammonia was added the solution became lighter in colour and gave sp. 4, Chart II. showing an undoubted origin from haematin; at the same time the band at F disappeared totally and was not replaced by another. These bands gave approximately the following readings: 1st (doubtful) λ 625 to λ 610, 2nd a shading beginning at about λ 597 and extending to the darker part or band from λ 585 to λ 559, 3rd λ 547 to λ 520.

With zinc chloride and ammonia a fine green fluorescence was seen and sp. 5, Chart II., which is drawn from a deep and shallow layer. The 1st band extended from about λ 647 to λ 625, then a shading commencing at about λ 608 and extending up to 2nd band from λ 587 to λ 562; that before F in a thinner layer in its darkest part read from λ 514 to λ 496, and was continued into a shading of about its own breadth towards violet.

With zinc chloride alone there was no distinct fluorescence, a feeble band was seen just after D, another in green and that at F remained untouched. The bands were difficult to measure and gave approxi-

[1] See below.

mately the following readings? 1st about λ 585 to λ 567·5, 2nd a shading λ 559 to λ 529, that at F remaining as before, from about λ 504 to λ 477, and being continued into a shading extending towards violet.

Hence this specimen evidently contained a pigment showing many points of resemblance to urohaematoporphyrin. Can this be explained on anatomical grounds? The first specimen was obtained from a case where the haemorrhage took place into the peritoneal cavity, the second where it took place into the leg.

The occurrence of urobilin (pathological) after blood extravasations has been noticed by various observers[1], and le Nobel[2] suggests that his urobilinoidin may have been mistaken for this, but in the first of the above cases it was not "urobilinoidin" but pathological urobilin that was present.

Urohaematoporphyrin and urobilin from a case of peritonitis. The urine was orange in colour and showed faint traces of the urohaematoporphyrin bands when treated with sulphuric acid. The colouring matter was obtained as before by precipitation with the lead acetates &c. The acidulated alcohol extract was red and showed the bands of acid urohaematoporphyrin, the band at F being dark, evidently due to mixture with pathological urobilin. The chloroform extract from this was light red by daylight and orange by gaslight. It gave a black band like that of pathological urobilin at F and some feeble bands in the red half of the spectrum. On evaporation it left a brownish residue.

This residue easily went into rectified spirit, forming an orange red solution by gaslight, and examined in a deep and shallow layer gave sp. 6, II.

With ammonia the colour became less red and the spectrum appeared as shown in sp. 7, wherein it is noticeable that the band at F was replaced by another nearer red, but this was very faint. The following readings of the bands produced by ammonia are approximately true: 1st λ 659 to λ 636, 2nd λ 625 to λ 612·5; a shading from λ 601 going up to 3rd from λ 585 to λ 555, 4th λ 542 to λ 523 and 5th a shading from λ 514 to λ 492(?). With zinc chloride and ammonia a well-marked green fluorescence was obtained, the bands appearing much as before, but after standing for a short time sp. 8, II. (mapped from a deep and shallow layer) appeared. Of which the bands read approximately: 1st about λ 653 to λ 630, then a shading at λ 597 extending up to 2nd λ 585 to

[1] E.g. Kunkel: *Virch. Archiv*, Vol. 81, and *Zeits. f. phys. Chem.* IV, V. Cf. Ziegler—Mac Alister: *Path. Anat.* Arts. 68, and 268.

[2] *Loc. cit.*

λ 569, 3rd λ 552 to λ 532 and 4th in thinner layer from λ 514 to λ 494 (= darker part, the band being faintly shaded to about λ 483). (Cf. with the 3rd reduction product of acid haematin *infra.*)

Zinc chloride alone produced a well-marked green fluorescence, and the spectrum produced was much the same as sp. 8, II., except that the first band was missing; the others read: 1st, in its darker part, λ 583 to λ 569, 2nd λ 550·5 to λ 532, 3rd, in thinner layer, dark from λ 517 to λ 499, continued into a shading extending towards violet. In every instance sodium hydroxide produced almost the same effect as ammonia; but owing to its having produced precipitation as a rule, I have not given the measurements of the bands.

The above examples will suffice to show the methods necessary for the detection of urohaematoporphyrin and for distinguishing it from pathological urobilin; and I have now to describe how the former can be prepared from haematin. To enable any one to perceive the connexion between the artificially prepared colouring matters and those which occur naturally in urine I shall postpone the consideration of normal urobilin, stercobilin, hydrobilirubin and biliary urobilin until these artificially prepared pigments have been considered.

Haematoporphyrin. First of all it was necessary to examine haematoporphyrin by the same methods as those just referred to, and to compare it with the other artificially prepared pigments. This is the more necessary as much confusion has crept into the descriptions of this colouring matter of late.

Haematin, prepared by the method described in this Journal[1], was dissolved in strong sulphuric acid and filtered through asbestos, the filtrate poured into water, and ammonia added until all the haematoporphyrin was precipitated, the precipitate being then well washed with water. It was then dissolved in rectified spirit acidulated with sulphuric acid, the resulting solution being deep red by gaslight in a deep layer and purple red in thinner layers. For a moderately diluted solution the following measurements were obtained: 1st band from λ605 to λ589, darker part from λ 603 to λ 591, then a shading beginning at λ 583 and extending to λ 566, where it became darker, the band extending to λ 538. This is shown in sp. 9, II. This spectrum differs in some respects from that of the first reduction product of haematin to be described further on. This solution was now agitated with chloroform, after diluting with water. The chloroform solution thus obtained was

[1] Vol. vi. Nos. 1 and 2. I followed Hoppe-Seyler's method in preparing haematoporphyrin.

a deep red colour and gave sp. 10, II., the bands reading: 1st λ 630 to λ 619, 2nd λ 610 to λ 593, 3rd, including shading, λ 587 to λ 545, where the band became dark and extended to about λ 526, 4th λ 517 to λ 490. On evaporating this solution on the water-bath, it left a dark brown amorphous residue, which in thin layers had a decidedly greenish tint by daylight. It was soluble in ether, chloroform, benzene, bisulphide of carbon, and rectified spirit. In the last with a red colour, but having a purplish tint in thin layers. A rectified spirit solution gave sp. 11, II.: the bands reading as follows: 1st λ 636 to λ 619, 2nd a feeble shading from about λ 612·5 to λ 593, 3rd λ 587 shaded, dark at λ 585, and from that to about λ 552, 4th a feeble shading from λ 545 to λ 526, and 5th about λ 515·5 to λ 490. With ammonia it became a much lighter red colour, and gave the four-banded spectrum of alkaline haematoporphyrin sp. 12, II.; the bands of such a solution reading: 1st from λ 630 to 617, then a lightly shaded part from λ 605 to λ 587, where it became darker and extended to λ 564, 3rd λ 552 to λ 529, and 4th λ 520 to λ 488.

Zinc chloride and ammonia produced a copious precipitate, which on filtering off left the solution almost colourless, the precipitate being a light brown colour. On getting it into solution again only the spectrum of alkaline haematoporphyrin was seen, no green fluorescence being present.

Zinc chloride alone produced no change in a rectified spirit solution.

Sodium hydroxide produced the same effect as ammonia.

It is quite evident then that haematoporphyrin is a different colouring matter from any of those I have described in the previous part of this paper, and differs from the reduction products of haematin described below.

Action of zinc and sulphuric acid on haematin. Haematin, prepared by the method mentioned, was dissolved in rectified spirit and sulphuric acid. At 4.10 p.m. sulphuric acid was added[1] and the action commenced at once, the fluid being heated on the water-bath. At 4.35 the bands of acid haematin were still visible, at 4.45 the 3rd haematin band from the red was getting darker, and the acid haematoporphyrin band at D becoming visible. At 4.50 the acid haematoporphyrin bands were fairly well marked. At 5 the band in red of acid haematin could hardly be seen, while in thin layers a band between green and blue was visible. At 5.15 the band in red of

[1] In a small flask provided with a perforated cork through which a tube passed to allow of the escape of gas, which came off freely.

acid haematin had gone, the fluid being a red colour (daylight). The fluid was then filtered. It had a deep purple red colour by daylight, and it had not yet reached the urohaematoporphyrin stage and yet the very well-marked 3rd band was seen. This is a different result to that obtained by le Nobel. The spectrum is shown in sp. 13, II. These bands read as follows: 1st from λ 601 to λ 585, darker from λ 597 to λ 589; then a shading beginning at λ 579 and extending to about λ 560·5 and dark (band) to λ 538; 3rd from about λ 529 to λ 469 (in a thinner layer). This solution was then agitated with chloroform. We may call the pigment in solution

(1) *The first reduction product*[1].

The chloroformic solution gave sp. 14, II., the result of examining a deep and shallow layer, the bands of which read as follows: 1st λ 653 to λ 641, 2nd λ 630 to λ 612·5 (dark from λ 625 to λ 615), 3rd band (or shading) λ 603 to λ 591, 4th λ 583 to λ 560·5 (= two bands), 5th λ 542 to λ 526, and in a thin layer 6th λ 514 to λ 473. The chloroform left on evaporation a brown residue, soluble in ether (in part), in rectified spirit, chloroform, &c. The rectified spirit extract gave the very beautiful spectrum shown in sp. 15, II., the bands of which read: 1st λ 627·5 to λ 615, 2nd λ 604 to λ 597, 3rd λ 594 to λ 590, then a shading from λ 585 to λ 573 where 4th band began, and it ended at λ 549, 5th λ 540 to λ 517, and 6th in a thinner layer λ 509 to λ 473.

With ammonia added to the rectified spirit solution sp. 16, II. was obtained: the bands of which read as follows: 1st λ 653 to λ 638·5, 2nd λ 625 to λ 612·5, then a feeble shading beginning at about λ 601 and extending to λ 585, where 3rd began, and this extended to λ 560·5, 4th in its darker part λ 538 to λ 526 (its shading began at λ 545 and extended to λ 523), 5th λ 515·5 to λ 485 (dark from λ 511 to 488). When treated with ammonia and zinc chloride the bands read just the same as with ammonia, but the fluid showed a green fluorescence. Zinc chloride alone did not develope a green fluorescence, although it did produce some slight shifting of the bands: thus they now read: 1st λ 627·5 to λ 615, 2nd λ 605 to λ 599, 3rd λ 595 to λ 587, 4th λ 581 to λ 567·5, 5th λ 557 to λ 549 (the whole shading of which this band formed a part reading λ 557 to λ 529), and 6th λ 509 to λ 473. Hence zinc chloride did not produce any narrowing of band at F.

[1] In calling these the 1st, 2nd and 3rd reduction products I do not mean to maintain that these were the only products formed. There may be any number of intermediate ones.

Sodium hydroxide produced the same effect as ammonia.

It is quite evident from a study of sp. 13, II. that this pigment had not yet reached the stage of urohaematoporphyrin. When the acid solution was first removed from the water-bath it was much lighter than when it had stood some time.

(2) *Second reduction product.*

The solution from which the above had been removed was at 3.25 next day again heated with zinc and sulphuric acid on the water-bath, At 3.55 the spectrum had not changed; at 4.30 the colour had become lighter: being then reddish-brown. Now the band at D and that in green were lighter than before, that at F being much darker, sp. 17, II. This spectrum shows that the pigment was further changed, which can be seen by comparing sp. 13, II. with sp. 17, II. These bands read: 1st λ 597 to λ 587, 2nd λ 557 to λ 543·5, 3rd, in a thinner layer, began to be feebly shaded at λ 529, dark at λ 514 and extended to λ 473. On agitating with chloroform the latter became orange with a tinge of purple, and showed a band in red besides the others, sp. 18, II. These bands read: 1st λ 630 to λ 617 (?), 2nd λ 601 to λ 589, 3rd λ 560·5 to λ 543·5, 4th, in thinner layers, λ 506 to λ 475 (in thicker layers a shading began at λ 526 and extended up to the last band).

This chloroform solution left on evaporation a sepia-brown amorphous residue, which when dissolved in rectified spirit formed a brownish-orange solution, showing sp. 19, II. (a combination spectrum). These bands read: 1st merged into the absorption of red end of spectrum, 2nd λ 625 to λ 612·5, then an uncertain shading before D, 3rd λ 583 to λ 560·5, 4th λ 540 to λ 523, and 5th in thinner layer λ 509 to λ 473, dark from λ 501 to λ 475. Now on comparing sp. 19 with sp. 15 a difference is apparent, although it is a very slight one, as the measurements show. With ammonia this rectified spirit solution became lighter in colour and gave sp. 20, II. (note the band nearest violet which is narrowed by the ammonia); the bands of which read: 1st λ 653 to 638·5, 2nd λ 625 to λ 612·5, 3rd λ 583 to λ 560·5 (with a shading before it), 4th λ 538 to λ 523, and 5th λ 514 to λ 490 (= darker part, the whole band reading λ 517 to λ 485).

Zinc chloride and ammonia left the bands in the same position as with ammonia, but the fluid had a faint green fluorescence, and the band nearest violet was darkened. With zinc chloride alone a rectified spirit solution had a purplish tint and did show an attempt at a

green fluorescence, and gave sp. 21, II. From which it is seen that now the pigment is coming to resemble the urohaematoporphyrin described above. These bands read:—1st λ 625 to λ 605 (?), 2nd λ 583 to λ 564, 3rd λ 549 to λ 529 (?), 4th (in thinner layer) λ 517 to λ 494 (= darker part, the whole band and shading from about λ 520 to about λ 473).

(3) *Third reduction product.*

The solution from which the last had been removed was again heated with zinc and sulphuric acid on the water-bath, as it had not yet reached the stage of urohaematoporphyrin, as can be seen by comparing the above results with those given in the former part of this paper.

The experiment began at 6.12 p.m. At 7.18 the fluid was of the colour of pale sherry, having evidently been completely reduced (and having reached the stage of urobilinoidin of le Nobel). It now showed a feeble shading at D and a dark band at the blue end of green. After standing exposed to the air the fluid became darker, until it became of an orange colour, in fact the same colour as a solution of urohaematoporphyrin; it then gave sp. 1, III. The first band seemed at first somewhat hazy, but afterwards became more distinct. The bands of the solution read:—1st λ 597 to λ 585, 2nd λ 557 to λ 542 and in a thinner layer 3rd band began to be feebly shaded at λ 523, dark at λ 504, dark up to λ 477, and shaded off to about λ 473.

When agitated with chloroform all the pigment went into solution, and when the chloroform was evaporated the residue was of a brown colour, having the peculiar smell which freshly prepared urobilin or urohaematoporphyrin has.

The rectified spirit solution was deep red in concentrated solution, by transmitted gaslight, red-brown in less deep layers, and gave sp. 2, III.; of which the bands read:—1st λ 653 to λ 638·5 merged into red end, 2nd λ 625 to λ 615, 3rd λ 603 to 564, 4th λ 542 to λ 523 (?) and 5th in a more dilute solution, began as a feeble shading at λ 511, darkened at λ 504, extended dark to λ 477, and shaded gradually off to about λ 473.

With ammonia the rectified spirit solution became lighter in colour (yellow with an excess) and sp. 3, III. appeared, which is evidently the spectrum of alkaline urohaematoporphyrin[1]; the bands then reading:—

[1] The band nearest violet is nearer the red than it appears in some cases.

1st λ 653 to λ 638·5, 2nd λ 625 to λ 512·5, then a shading commencing about λ 601 and extending to 3rd band from λ 583 to λ 562, 4th λ 540 to λ 526 and 5th in a thinner layer λ 517 to λ 494, while in a still thinner layer it read λ 514 to λ 494. Comparing these measurements with those of the pigments of pathological origin, there cannot be the shadow of a doubt that here urohaematoporphyrin had been artificially produced, and I would lay particular stress on the band nearest violet; for we see that whereas in the case of pathological urobilin it may be absent or replaced by a mere shadow, here it is tolerably dark.

The action of zinc chloride and ammonia was no less characteristic. The solution under the influence of these reagents was reddish yellow; it showed a green fluorescence and gave sp. 4, III., of which the bands read:—1st λ 653 to λ 638·5, 2nd λ 625 to λ 612·5, then a shading beginning at about λ 601 and extending to 3rd band, from λ 585 to λ 562, 4th dark from λ 517 to λ 494 and then gradually shading off towards violet. The last band corresponds also with that of a similar solution of the pigment of pathological origin. Some slight differences exist between the feeble bands, but these are of no consequence.

Zinc chloride alone produced—as it does with the corresponding solution of the pathological pigment—darkening of the band at D and intensification of that nearest violet sp. 5, III., moreover the fluid possessed a greenish fluorescence. The bands of this solution read:—1st λ 625 to λ 607·5, then a feeble shading from about λ 597 up to λ 585, 2nd band λ 585 to λ 566, 3rd λ 549 to λ 532, 4th dark part λ 517 to λ 494, shading gradually off towards violet.

It would therefore appear that the band at F in acid solution, the green fluorescence with zinc chloride and ammonia, the shifting of band at F towards red with ammonia, are as characteristic of urohaematoporphyrin as of urobilin, and there appears to be no valid reason for concluding that we must assume the existence of a new pigment—"urobilinoidin"—in this case[1].

Reduction of Haematin with Sodium Amalgam.

Haematin prepared by the method referred to above was dissolved in rectified spirit containing a little aqueous soda and filtered. This gave the spectrum of alkaline haematin. Sodium amalgam being then introduced into the flask (which was provided with a tube run through the cork) it was gently heated on the water-bath.

[1] This being merely the chromogen, or further reduced condition of urohaematoporphyrin.

The experiment began at 9.10[1]; at 9.25 a narrow band had appeared in the green and one at the blue end of green darker than the former, but still the alkaline haematin band was present. At 9.29 the second band was darker. At 9.38 the band of alkaline haematin at D was seen to be changing and another began to appear in the extreme red, the band between green and blue being the darkest of all. At 9.45 the fluid gave sp. 6, III., which closely resembled urohaematoporphyrin, but it had not really reached that stage. We may call the pigment in solution at this stage the

(1) *First reduction product.*

The dark red solution, from which sp. 6, III. was mapped, gave the following bands:—1st λ 665 to λ 644, 2nd λ 636 to λ 617, 3rd λ 607·5 to λ 581, 4th λ 549 to λ 527·5 and 5th in a thinner layer λ 515·5 to λ 490 (?).

Sulphuric acid was now added to acidity, when the fluid became a bright red colour and gave sp. 7, III., from which we see that even at this early stage a band at F was present, i.e. the stage prior to the formation of true urohaematoporphyrin, so that here again one can prove that le Nobel's statement is not correct, as he says the band at F does not occur until after this stage. These bands read:—1st λ 644 to λ 619 (?), 2nd λ 607·5 to λ 591, 3rd λ 566 to λ 538 (?) and 4th about λ 506 to λ 479 (= darker part).

This solution was then agitated with chloroform (after dilution with water): the chloroform became purple-red, took up all the pigment except a trace, and gave sp. 8, III.; of which the following are the measurements of the bands:—1st λ 665 to λ 622, 2nd λ 607·5 to λ 593, 3rd feeble shading λ 585 to λ 577, 4th λ 567·5 to λ 542, and 5th began to be feebly shaded at about λ 523, then became dark at λ 506 and was dark to about λ 479.

On evaporating the chloroform it left a violet-brown residue, which dissolved in rectified spirit with a purplish tint, and gave sp. 9, III., the bands of which read:—1st λ 636 to λ 615, 2nd feebly shaded at λ 589, dark at λ 581 and extended to λ 564, 3rd λ 547 to λ 524·5, and 4th λ 517 to λ 483 (?).

With ammonia the solution became orange red and gave sp. 10, III. of which the bands read:—1st λ 665 to λ 641 (?), 2nd λ 636 to λ 617, 3rd

[1] From time to time the action may have been lessened owing to my having to examine the spectrum.

a shading beginning at λ 607·5 and extending to the darker part from λ 585 to λ 562, 4th λ 545 to λ 526, and 5th about λ 514 to λ 490.

With ammonia and zinc chloride there was an attempt at a green fluorescence, the bands reading:—1st λ 662 to λ 641, 2nd λ 636 to λ 617, 3rd a shading from λ 610 and extending to darker part from λ 585 to λ 562, 4th λ 547 to λ 429 and 5th λ 524 to λ 490 (?). Therefore the addition of these reagents produced no marked change.

With zinc chloride alone sp. 11, III. was produced, showing that some change had taken place (this is evident on comparing this spectrum with sp. 9, III.). These bands read:—1st λ 653 to λ 619 (?), 2nd a shading at about λ 595, the band becoming dark at λ 589 and extending to λ 571, 3rd λ 555 to λ 529 (?) and in a thinner layer 4th λ 514 to λ 481. This last band is quite different from the corresponding one of urohaematoporphyrin.

(2) *Second reduction product.*

The solution from which the above had been removed was further reduced with sodium amalgam until the solution showed sp. 12, III. It got much redder after standing exposed to the air. The bands shown in sp. 12 read:—1st λ 665 to λ 641 (?), 2nd λ 636 to λ 615, 3rd λ 601 to λ 566, 4th λ 555 to λ 526 including shadings, and 5th λ 517 to λ 490. The fluid was now treated with sulphuric acid to acidity (reserving a part for further reduction). It was filtered from the sodium sulphate, and had a red colour with a faint tinge of purple and gave sp. 13, III. The feeble shading of band nearest violet was remarkable. The 2nd very narrow band is also worthy of notice, as I had previously seen it in a similar solution of urohaematoporphyrin from Addison's disease and acute rheumatism[1]. These bands read: 1st λ 607·5 to λ 591, 2nd about λ 583 to λ 573, 3rd λ 566 to λ 538, 4th uncertain. After dilution with water the solution was now agitated with chloroform, which assumed a reddish yellow colour, and gave the same bands as sp. 13, only altered in position. On evaporating the chloroform a brown residue with a purplish tint was left, but by daylight it had a brownish green colour. It was partially soluble in ether, and in rectified spirit. The rectified spirit solution had a purplish tint and gave sp. 14, III., from which it appears that the colouring matter had not yet reached the urohaematoporphyrin stage. The bands read as follows:—1st

[1] See this *Journal*, Vol. VI. Nos. 1 and 2, p. 36, 3rd line from bottom of page. Also Vol. VII. No. 3, Pl. VIII. and Vol. VIII. No. 6, Pl. XI.

λ 633 to λ 619, 2nd about λ 610 to λ 599, 3rd about λ 585 to λ 550·5, 4th λ 543·5 to λ 521·5, and 5th λ 514 to λ 488 (?).

Ammonia made this solution a lighter red colour, and produced a spectrum closely resembling alkaline haematoporphyrin, of which the bands read:—1st λ 633 to λ 615, 2nd in its dark part λ 585 to λ 562 with an ill-defined shading before it, 3rd λ 545 to λ 526, and 4th about λ 514 to λ 488.

With zinc chloride and ammonia the bands remained practically the same, so that the urohaematoporphyrin stage had not been yet reached, as stated above.

(3) *Third reduction product.*

After half-an-hour's further action the fluid showed a new spectrum, and on standing exposed to the air it assumed an orange colour and gave sp. 15, III. The shading before D began at about λ 625, the band beginning to be darker at λ 597 and extending to about λ 573, 2nd a faint shading from about λ 562 to λ 529, and 3rd about λ 514 to λ 490.

On acidulating with sulphuric acid until the reaction was decidedly acid the bands read: 1st λ 605 to λ 591, 2nd about λ 562 to λ 545, 3rd in its dark part λ 501 to λ 473, but these measurements were uncertain. On agitating with chloroform the latter became yellow and gave sp. 16, III. with the above measurements, differing slightly owing to the nature of the solvent. On evaporating the chloroform a brown residue was left which had the same smell as freshly prepared urobilin.

The rectified spirit extract of this residue was reddish or yellowish red, by transmitted gaslight; a thin layer showing a dark band at F and a thicker layer sp. 17, III. The following are only approximate measurements of the bands, owing to the difficulty of reading them: 1st band invisible, 2nd λ 605 to λ 591, 3rd λ 562 to λ 549, 4th λ 506 to λ 479 (= darker part).

With ammonia precipitation was produced; after filtering sp. 18, III. was obtained, which if belonging to alkaline urohaematoporphyrin wants one band in the red: these bands read:—1st λ 633 (or 636) to λ 617, then a shading at λ 605 (?), 2nd band began at λ 587 and extended to λ 562, 3rd λ 547 to λ 529, and 4th λ 514 to λ 490.

When an alcohol solution was treated with ammonia and zinc chloride, the 1st, 2nd and 3rd bands remain much the same as before, but the 4th band was markedly intensified; and a faint green fluorescence was perceptible.

Zinc chloride alone did not cause the band at F to move towards the red nor was any very distinct fluorescence perceptible. Hence reducing agents differ in their action on haematin, for although urohaematoporphyrin was produced by sodium amalgam, yet its spectra differed slightly from those of the pigment produced by zinc and sulphuric acid. I also tried the action of zinc dust on alkaline haematin and satisfied myself that a band appeared at F before the urohaematoporphyrin stage was reached, but with this reagent also other products of reduction were formed, differing in some respects from those formed by the above reducing agents.

Before leaving this subject I should like to remark once more that an inspection of le Nobel's plate and a comparison of that with mine will clearly show that his isohaematoporphyrin was not the same colouring matter as that which I at one time called "urohaematin," what he took for this substance was a product of reduction which had not yet reached the urohaematoporphyrin stage.

Normal Urobilin.

I may now describe normal urobilin and show how it differs from pathological urobilin, urohaematoporphyrin and other colouring matters to be described further on. In the paper referred to at the beginning of this, I said that I had prepared an identical substance by the action of peroxide of hydrogen on acid haematin. Respecting this le Nobel remarks:—"Denn auch die Angabe MacMunn's nach welcher es gelingt aus Haematin mittelst Wasserstoffsuperoxyd Urobilin darzustellen, bedarf noch sehr einer genauen Nachprüfung, und ist meiner Meinung nach mindestens zweifelhaft." It is not at all "zweifelhaft," and it has received a "genauen Nachprüfung," as I shall show further on. In the first experiment some litres of normal urine were treated as before with the lead acetates, acidulated alcohol and chloroform.

The acidulated alcohol extract of the lead precipitate was a dull brown or yellow brown, not the red brown of pathological urobilin; it gave a band at F with ill-defined edges, and no matter how deep the layer of fluid this band never appeared broad and black like the band of pathological urobilin. A deep layer showed a faint haze in orange; the band at F read from λ 502·3 to λ 481, but its edges gradually thinned out, so that it was not easy to say where it began or ended, and it had not a less shaded part extending towards the violet. For comparison with other solutions I have mapped the spectrum of a deep and shallow layer of this acidulated alcohol solution: sp. 1, and

2, Chart IV. After standing exposed to the air this solution became redder, owing to the oxidation of a chromogen or chromogens.

On diluting with water and agitating with chloroform this became yellow, although in deep layers it seemed orange-yellow, and it left on evaporation a residue which was brown in thicker parts, and yellow in thinner ones.

On dissolving the residue in alcohol, a red solution (by gaslight) was obtained; of which a deep layer transmitted red and a little green; a shallow one gave an ill-defined band at F. I fancied I could perceive a faint band before D from λ 607·5 to λ 593 (?), that at F read λ 504 to λ 477. Sp. 3, IV. represents the latter band.

Treated with ammonia and filtered a deep layer seemed to give a very faint band in red sp. 4, IV. but there was no band after D, and the band at F had disappeared *in toto* or left the faintest possible shadow.

With zinc chloride and ammonia a yellow solution with a fine green fluorescence was obtained, giving sp. 5, IV. The bands were faint, so that the following measurements are only approximate:—1st λ 650 to λ 625, 2nd in its darker part λ 587 to λ 571, with a shading before it, 3rd (darker part) λ 514 to λ 496, and this had not the same kind of shading as the band of pathological urobilin, as it did not shade off towards violet like it.

Sodium hydroxide in excess caused precipitation, but after filtering some new bands appeared, which read approximately: 1st λ 653 to λ 625, then an uncertain one at D, and 3rd λ 514 to λ 496.

On standing exposed to the air the band at F seemed to become darker: this I believe must have been due to the presence of a second chromogen, as I formerly inferred, probably the chromogen of pathological urobilin, or what is the same thing, that of stercobilin. Therefore in operating on normal urobilin one should carry out its separation as quickly as possible.

In a second experiment the acidulated alcohol showed exactly the same spectra as before; after the evaporation of the chloroform extract of this, a brown amorphous residue was left. The alcohol solution of this residue was of a brown colour, more red by gaslight; in a deep layer I fancied I could see some shading before D, in a thin layer it showed a band at F from λ 504 to λ 477, and this had the same characters as those referred to above.

With ammonia no bands could be seen, with it and zinc chloride the fluid did not show much fluorescence, this being a dull green. Shifting of the band at F took place, but this band did not show a less shaded

part extending towards F; but in the red and at the commencement of green some feeble bands were visible: all these read: 1st λ 650 to λ 625, 2nd (darker part) λ 587 to λ 571, 3rd λ 514 to λ 496.

Zinc chloride alone did not cause a green fluorescence, and did not affect the band at F; in my former results[1] I found however that it did affect the band. It would appear therefore that too much stress should not be laid on this reaction. What is of more importance is the absence of green fluorescence, but possibly that too may vary. Sodium hydroxide caused the band at F to disappear, but with an excess a faint shading was seen replacing it from λ 514 to λ 496. I missed this in my former experiments (in 1880) owing to the use of less perfect spectroscopes.

Sometimes we come upon normal urobilin which shows more resemblances to pathological urobilin than the above. As I remarked in the paper quoted above[2], "The pigment may sometimes appear more brown than brown yellow in colour, and in that case it shows a tendency to imitate febrile urobilin in its behaviour with the caustic alkalies, for on their addition a feeble band may be noticed nearer the red than the original band.....The colour depends upon the amount of oxidation to which it has been subjected in the body, as well as on its artificial preparation."

The following experiment illustrates the necessity of separating normal urobilin quickly. Normal urine was treated as before. The acidulated alcohol extract of the lead precipitate was at first a dull, then a deep yellow; and the solution was let stand for several hours, so that possibly the other chromogen referred to above may have become oxidised; still the band at F was not like that of pathological, but resembled that of normal urobilin.

The chloroform extract was dull yellow and showed a faint red tint where its edge touched the white dish (as I showed in the paper quoted above). The residue from this solution was brown, showing no trace of red. The rectified spirit solution was brown, being a dark yellow in thinner layers with transmitted daylight. It showed some shading in green and the band at F from λ 504 to λ 479. When ammonia was added to this some feeble bands too faint to be measured were just visible, viz. a feeble shadow before D, another after D and a third near E. With zinc chloride and ammonia a faint green fluorescence was seen, and now a band or rather shading appeared in the red, another at D, and the band at F was replaced by another nearer the red, the last from λ 517 to λ 494.

[1] *Proc. Roy. Soc. loc. cit.* [2] *Proc. Roy. Soc. loc. cit.*

Zinc chloride alone produced sp. 6, IV., showing as before, that the band at F had not moved towards red. Approximately these read:— 1st λ 583 to λ 571, 2nd λ 549 to λ 523? and that at F λ 506 to λ 479. Here evidently the second chromogen was present.

Action of Peroxide of Hydrogen on Acid Haematin. Acid haematin prepared by acting on sheep blood with rectified spirit acidulated with sulphuric acid (1 in 17) and filtering, without further preparation was treated with freshly prepared peroxide of hydrogen until the acid haematin bands had gone, leaving a band between green and blue from about λ 504 to λ 481. It was then agitated with chloroform in a separating funnel; the chloroform became reddish yellow, or orange yellow, with a reddish tint where it touched the side of the white dish, and showed a distinct band from λ 504 to λ 481, looking exactly like the band of normal urobilin. It was then evaporated and left a brown pigment having a peculiar smell recalling to mind that of freshly prepared urobilin; this was soluble in the solvents of normal urobilin, but owing to the faintness of the band at F, I proceeded to slightly reduce it with sodium amalgam. A rectified spirit extract was diluted with water and placed in a small flask with an exit tube and sodium amalgam added. After a little time it became orange red in colour and then showed two bands placed closely together, which read: 1st λ 543·5 to 526, 2nd λ 521·5 to λ 501 (?). It was then treated with sulphuric acid to acidity and became red in colour; a deep layer absorbed all the violet end of the spectrum up to near D, while in a thin layer a dark band became detached from about λ 504 to λ 475. The chloroform extract from this showed a band from λ 504 to λ 481 (= darker part) and had a red colour. On evaporation this left a deep brown residue which had the peculiar smell noted above. It dissolved readily in rectified spirit, forming a reddish-brown solution; in a deep layer transmitting only the red rays, in less deep it showed a very ill-defined shading near D, while in shallower layers still, a band was seen at F, shown in sp. 7, IV., which read from λ 504 to λ 479.

With ammonia no band at F or elsewhere could be seen.

With ammonia and zinc chloride a well-marked green fluorescence was obtained and sp. 8, IV. appeared, and this showed a most remarkable likeness to normal urobilin even to the feeble band in red which probably read from λ 647 to λ 625 (or λ 650 to λ 625), the other from λ 517 to λ 496 or λ 494, also corresponding to that of normal urobilin[1]. The fluorescence too was a dull green as in the case of the latter.

[1] The band after D was however missing, but it is probably not constant in the spectrum of normal urobilin.

Zinc chloride alone however shifted the band at F nearer the red, from λ 517 to λ 494. This was owing doubtless to my having reduced the pigment too much, as its action showed it was approaching to the stage of pathological urobilin.

Sodium hydroxide produced a similar band.

I am convinced then from this experiment that normal urobilin is mostly a metabolite of haematin produced in the organism by the action of nascent oxygen on haematin.

Stercobilin. I have already in this Journal[1] described the spectrum of stercobilin, but at that time I was not aware of the importance of the feeble bands in its solutions, and missed most of them, for in spectroscopic analysis as well as in telescopic observation having once seen a thing one recognises it with greater readiness again. In the case of stercobilin, as in that of other colouring matters, we meet with the pigment in various stages of preparation, so that its spectra vary slightly. It was prepared in the same manner as that described in my former paper[2]. An acidulated filtered alcohol extract (17 parts of rect. spt. to 3 H_2SO_4), after dilution with water, being shaken with chloroform and the latter evaporated on the water-bath. The acidulated spirit extract was of a very deep red colour (transmitted gaslight), showing in a deep layer a band covering D, and in a less deep a band at F. A very feeble shading began at λ 625, got darker at λ 610 and extended to about λ 569, in a deep layer, while in a shallower one it read from λ 610 to λ 573; a feeble shading in green was also seen, and a band at F, from about λ 506 to λ 479.

The extract obtained by agitating this solution with chloroform was of an orange colour, and gave three feeble bands and a fourth dark one, all reading:—1st λ 612·5 to 591, 2nd λ 589 to λ 567·5, 3rd λ 550·5 to λ 529, and 4th in its darker part λ 506 to λ 481. On evaporation this left a brown residue.

Dissolved in rectified spirit this furnished a reddish-brown solution showing a band at D, and one at F, sp. 9, IV. which read: 1st shaded at λ 617, dark at 607·5 to 577, where it gradually shaded off to λ 569, 2nd, dark part λ 504 to λ 479, whole band λ 509 to λ 475.

Treated with ammonia sp. 10, IV. appeared.

These bands read approximately:—1st λ 636 to λ 619, (?) then a shading at about λ 610 extending to 2nd band λ 587 to λ 562, 3rd λ 549 to λ 526, and in a thin layer 4th λ 517-λ 494? So that ammonia,

[1] This *Journal*, Vol. VI. Nos. 1 and 2, p. 31 and following.

[2] *Loc. cit.*

as in the case of urohaematoporphyrin, caused the band at F to be replaced by another nearer the red end of the spectrum. With ammonia and zinc chloride a green fluorescence appeared and the bands shown in sp. 11, IV. (which was mapped from a deep and shallow layer of fluid). These read: 1st λ 656 to λ 622, and dark from λ 653 to λ 630, 2nd commenced as a shading at λ 607·5 extending to λ 593, where it got darker and extended to λ 569, 3rd λ 555 to λ 532, and 4th λ 514 to λ 496, shaded off to λ 488.

Zinc chloride alone developed a fine green fluorescence and the bands seen in sp. 12, IV., appeared, reading:—1st, a shading commencing at λ 607·5, extending to the band: λ 591 to λ 569, 2nd, a shading λ 556 to λ 532?, 3rd in shallow layer, λ 515·5 to λ 499 shading off to about 483. Sodium hydroxide caused a band to appear from about λ 653 to λ 625, then an ill-defined shading beginning at about λ 610, darker at λ 593 and shaded off to about λ 569, 3rd a shading, perhaps λ 549 to λ 529, and 4th in a thinner layer λ 520 to λ 499. Hence this colouring matter resembles pathological urobilin very closely. In fact it may be considered identical with it.

In the next experiment there was a trace of some extraneous colouring matter present, perhaps chlorophyll from food, still I shall give the results as the influence of this chlorophyll (?) may be allowed for. The acidulated alcohol extract was a wine-red colour and gave sp. 13, IV., which is the result of combining the spectra of three different layers of fluid. These bands read:—1st λ 671 to λ 647, 2nd λ 636 to λ 615, 3rd λ 562 to λ 538, and 4th in a thinner layer λ 504 to λ 477. On diluting with water and agitating with chloroform an orange coloured solution was obtained. On evaporating this a brown residue was left soluble in ether, alcohol, chloroform, partially in bisulphide of carbon and benzene. It is unnecessary to map the spectra of all these solutions, as their bands were the same as in the alcohol solution, being simply changed in position by the nature of the solvent. The rectified spirit extract of the residue was orange-brown in colour (by daylight, and red by gaslight); in a deep layer giving a very broad band at D and in shallower a black one at F. The shading of that before D began at λ 636, it became darker at λ 610 extended dark to λ 577, and shaded off to λ 566; that at F began to be shaded at λ 514, was black at λ 505 up to λ 479 and shaded off to about λ 473. I cannot explain how it was that this rectified spirit solution differed in spectrum from the other solutions, such as chloroform, and benzene, as all the residue was soluble in spirit.

When a rectified spirit extract was treated with ammonia the same spectrum as sp. 10, IV., and giving the same readings appeared, the band at F being replaced by a shading nearer red.

With zinc chloride and ammonia, sp. 11, IV. was seen, of which the bands read:—1st about λ 653 to λ 625, then a shading about λ 610, extending to 2nd band λ 593 to λ 569 (?) 3rd λ 552 to λ 529 (?) and 4th in its darker part λ 515·5 to λ 496, gradually shading off to λ 483 (?).

With zinc chloride alone a fine green fluorescence appeared, and the spectrum of a deep and shallow layer is shown in sp. 14, IV. The readings were· shading at λ 615, extending to 1st band λ 593 to λ 569, 2nd band about λ 559 to λ 532 (?) 3rd in its dark part λ 518·5 to λ 496 and shaded off to λ 483 or beyond it.

Sodium hydroxide developed bands very like the last but with an additional one in red sp. 15, IV., these read approximately:—1st λ 653 to λ 625, then a shading at λ 610 extending to λ 597, where it got darker and extended to λ 569, 3rd λ 552 to λ 532, and 4th in thinner layer from λ 518·5 to λ 499 in its darker part; gradually shading off to about λ 483. These results therefore confirm the former ones.

Stercobilin therefore possesses in acid solution a black band at F like that of pathological urobilin, it shows in the feeble bands produced by various reagents an undoubted parentage—in part at least—from haematin. It differs markedly from normal urobilin, from urohaematoporphyrin, and as I shall now show from hydrobilirubin. It gives like pathological urobilin a green fluorescence, with zinc chloride alone, and its band at F moves towards the red with that reagent, this band being shaded off towards violet as in the case of pathological urobilin. In fact as I formerly stated there is no difference worth mentioning between these two pigments.

Preparation of Hydrobilirubin.

I have now to show that hydrobilirubin is not identical with any of the above colouring matters, but bears a very remarkable resemblance to a body present in bile, which I formerly[1] named the "urobilin of bile." I have prepared hydrobilirubin several times and each time arrived at the same conclution.

Pure bilirubin was in all the following experiments used for preparing hydrobilirubin. This bilirubin was thus prepared: Brown human gallstones were finely powdered, extracted with ether, boiled

[1] *Proc. Roy. Soc. loc. cit.*

with water, then treated with dilute hydrochloric acid, washed with water, dried, extracted with chloroform, the filtered chloroform extract evaporated down, the residue extracted with ether, then with alcohol, the residue left after the extraction by the latter solvent dissolved in chloroform; this was concentrated by evaporation, and as it went down absolute alcohol was added which precipitated out the bilirubin as a fine orange-coloured powder.

Instead of merely suspending the bilirubin in water I dissolved it in a weak solution of sodium hydroxide, introduced it into a small flask provided with an exit tube, added some sodium amalgam and gently heated it on the water-bath. Owing to the very abundant frothing I had to add a little absolute alcohol. One hour after the beginning of the experiment the solution had become a dull brownish colour, and then it had lost its strong absorptive power for the violet end of the spectrum. When a little was removed from the flask and hydrochloric acid added it became a kind of green colour and showed a band at F. After one hour and 12 minutes had elapsed (from the beginning of experiment) the fluid had become a dirty brown-yellow. After two hours and five minutes it had a dirty yellow tint, and floating on the surface was some dark-colouring matter, evidently a bye-product. On filtering, the filtrate became much darker (and on shaking some with air it became darker still); on the filtering paper some dark blue-green deposit was left (A). The filtrate was a kind of olive-brown-yellow (gaslight). Examined with the spectroscope it showed in a deep layer two bands: one in red, another just after D and a feeble shading at the blue end of green. On adding hydrochloric acid the colour became much darker, being reddish by transmitted, but having a bluish tint with reflected gaslight. And it then gave a spectrum like that of the Gmelin-Heintz reaction at an early stage; (sp. 16, IV.) at that stage when the band after D is just beginning to appear. This is very important, as it shows that here the change is not due to reduction, but to oxidation, or to a splitting up of the pigment. These bands read: 1st λ 619 to λ 581, then a shading after D, 3rd λ 506 to λ 481. A precipitate was produced in this solution by the hydrochloric acid, which is generally supposed to be hydrobilirubin since Maly first described it[1]. This precipitate (B) was filtered off, it had a dark bluish green colour. From the colour of this, evidently the pigment had not undergone sufficient reduction, but as the results obtained by the examination of

[1] *Ann. Chem. Pharm.* Bd. 161, S. 368, and Bd. 163, S. 77.

this and of the deposit (A) led to interesting results I think it necessary to give a description of them.

Disqué[1] indeed asks the question "Wie lange sollte nun reducirt werden, um reines Urobilin zu erhalten?" And he then adds "Nach Maly so lange bis die Flüssigkeit rothbraun sei und sich nicht mehr heller färbe." The fluid from which (B) was separated was a fine red-violet by transmitted gaslight. On agitating it with chloroform the latter assumed a violet colour, and then showed three bands evidently corresponding to those which occur at the violet stage of the Gmelin-Heintz reaction, and which resemble in many respects those of the bilicyanin of Heynsius and Campbell[2]. One before, another after D, and one at F; these read approximately: 1st λ 622 to λ 597, 2nd about λ 577 to λ 542, 3rd λ 509 to λ 481. (Sp. 17, IV.) On evaporating this chloroform solution on the water-bath a dark brown residue (C) was left. It became slightly changed by heating, as anyone can see who repeats this experiment.

I shall now describe in detail the results of examining each pigment separately. But I may remark that Disqué is correct when he says: "Es scheint sich also ein Zwischenprodukt zu bilden, welches dann in Hydrobilirubin übergeführt wird, bei der weiteren Behandlung mit Natriumamalgam wird aber das Hydrobilirubin selbst verändert, so dass es nicht wohl möglich ist durch diese Reduction allein einen reinen Körper zu erhalten." Further he says: "Die ganze dunkle fast schwarze Chloroformlösung wurde auf Uhrgläsern durch Stehen an der Luft verdunstet. Es blieb ein dunkler grün metallisch glänzender Körper zurück."

(C) This residue went easily into absolute alcohol, forming a brown-violet solution; with transmitted gaslight it had a deep red colour. It gave a band just before D, another between D and E and a dark one at F: reading: 1st λ 622 to λ 589, 2nd λ 569 to λ 538 and 3rd λ 506 to λ 479. Treated with ammonia this solution became of a lighter colour and then two new bands appeared, that at F having totally disappeared, these are shown in sp. 19, IV. With zinc chloride and ammonia a green solution with a red fluorescence was obtained giving sp. 20, IV. This is remarkable for two reasons:—(1) its remote likeness to chlorophyll, (2) its resemblance to a similar body which I shall show to be present in human bile. These bands read as follows: 1st λ 665 to λ 615, and dark from λ 659 to λ 627·5, 2nd λ 603 to λ 571, and 3rd λ 517 to

[1] *Zeits. f. physiol. Chem.* II Band, 4 Heft. 1878, S. 263.
[2] Cf. Le Noble, *loc. cit.*

λ 490, and darker part λ 512·5 to λ 496. With zinc chloride alone the colour of a rectified spirit solution became a kind of greenish brown with a greenish fluorescence and similar bands to the last-described were seen: sp. 1, chart V. In a deep layer these read: 1st λ 668 to λ 622 and darker from λ 662 to λ 630, 2nd λ 603 to λ 571, and in a thinner layer 3rd, λ 521·5 to λ 496, and dark part λ 515·5 to λ 501. The last, too, was shaded off towards violet like the band of pathological urobilin and stercobilin.

With sodium hydroxide a rectified spirit solution became a kind of bronze colour and gave on standing sp. 2, V. of which the bands read approximately: 1st λ 668 to λ 627·5, 2nd an uncertain shading, 3rd, λ 514 to λ 499.

(A) This was the deposit which was obtained on filtering the solution after it had been acted upon with the sodium amalgam; it was insoluble in absolute alcohol, ether, chloroform, and in cold rectified spirit, but on boiling with the latter a little went into solution with a greenish yellow colour. In rectified spirit acidulated with sulphuric acid it dissolved, forming a purple solution which showed a band before and another after D, but did not show a dark band at F. Hence this was merely a changed bile-pigment. It also dissolved in rectified spirit containing sodium hydroxide, but in this it gave no bands.

(B): the precipitate which formed in the solution after adding hydrochloric acid (which according to Maly is hydrobilirubin) was a dark dirty blue-green powder. It dissolved in absolute alcohol forming a very dark green solution. Owing to the loss of colouring matter, Disqué[1] abbreviated Maly's process by dissolving the precipitate in alcohol, then adding chloroform, acid and much water, and shaking the mixture. Accordingly, I diluted the solution with water, added some hydrochloric acid and then shook with chloroform in a separating funnel. The chloroform assumed a fine green colour; this solution was left to evaporate at the temperature of the air and finally left an amorphous residue which on a watch-glass appeared with transmitted day- and gas- light green in its thinner parts, but almost black in thicker parts.

A portion went into absolute alcohol, forming a dark green solution showing only one discernible band, that at F from λ 504 to λ 485 (?). With ammonia the colour became a lighter green, and then showed an

[1] *Loc. cit.* S. 264.

ill-defined shading in red, the band at F being gone. With zinc chloride and ammonia the green colour became lighter and now a red fluorescence was seen and the following bands: 1st λ 662 to λ 619, 2nd λ 603 to λ 573, and 3rd (about) λ 515·5 to λ 496—sp. 3, V. With zinc chloride alone the colour remained green and yet the solution showed some green fluorescence and the following bands: 1st λ 662 to λ 622, 2nd about λ 601 to λ 571, 3rd (darker part) λ 517 to λ 504, sp. 4, V.

Now it was quite evident that the action of the sodium amalgam had not gone on long enough in this experiment, but still the experiment was important in showing that sodium amalgam produces substances which are identical with those which arise during Gmelin's reaction; one of which gives a red fluorescence and a band in red, and is identical with or nearly related to a substance which occurs naturally in bile—as will be shown further on.

In the next experiment the bilirubin was prepared as before, and dissolved in a weak solution of sodium hydroxide to which some rectified spirit was added to prevent frothing. It was then heated on the water-bath. At first the colour became darker, but after two hours' action it had assumed a brownish-yellow colour and the solution was then filtered; on filtering it became much darker; being a deep dark red, by transmitted gaslight. A very small quantity of a dark green bye-product (A) was left on the filtering paper. A moderately deep layer of the filtered solution gave sp. 5, V., the fourth band of which became better marked after standing. These bands read as follows:—1st λ 644 to λ 619, 2nd λ 605 to λ 573, 3rd λ 555 to λ 532, (?) 4th (in a thinner layer) λ 514 to λ 494. This solution was then acidulated with hydrochloric acid, when it became very much darker and gave a band covering D, while a band became detached at F with its darker part from λ 504 to λ 475. A small quantity of a precipitate (B) formed, which after filtering appeared on the filtering paper as a greenish powder. The acid filtrate after dilution with water was shaken with chloroform in a separating funnel. The chloroform became brown-violet in colour, and on evaporation on the water-bath left a dark brown residue (C) which by daylight had a brown-violet tint. This residue had a peculiar smell, reminding of freshly prepared urobilin. The chloroform solution of this residue was violet-red in colour and gave sp. 6, V.; it differs from sp. 17, IV., in the position of the second band, but this was due to the fact that in the present instance the pigment had become more changed than in the former one. These bands read:—1st

λ 619 to λ 591, 2nd λ 587 to λ 545, 3rd in a thinner layer, λ 506 to λ 481 (= dark part). I now proceed to give the results of an examination of the pigments (C), (B), and (A).

(C) The rectified spirit extract of (C) was a deep red by transmitted gaslight and did not give two bands at D, but one, sp. 7, V.; the bands of which read: 1st λ 610 to λ 549, including shadings, 2nd in a thinner layer began to be shaded at λ 511, became black at λ 504 up to λ 477, and shaded gradually off to λ 469.

With ammonia a band appeared at D and that at F disappeared. With ammonia and zinc chloride the colour of the solution appeared bronze-green or brownish and showed a dull reddish fluorescence, and gave sp. 8 and 9, V; the bands of which read: 1st λ 653 to λ 607·5, 2nd λ 601 to λ 560·5 (in a concentrated solution), then in a thinner layer black part of 3rd band λ 517 to λ 494, where it shaded gradually off to about λ 477. Zinc chloride alone did not change the colour of the solution although it developed a green fluorescence; this is noteworthy, for zinc chloride with ammonia developed a reddish one, and some bands appeared resembling those of 9, V., very closely, only differing in the position of 1st and 2nd; thus they read 1st λ 647 to λ 617, 2nd λ 599 to λ 559, dark part λ 587 to λ 566, 3rd λ 520 to λ 494 (= dark part), gradually shading off to violet (to about λ 473).

Sodium hydroxide changed the colour of a rectified spirit solution to yellow with a tinge of green (daylight) and developed a spectrum resembling sp. 10, V., only that the bands were slightly altered in position. They read: 1st λ 650 to λ 617, 2nd λ 601 to λ 566, 3rd (in thinner layer) λ 517 to λ 494 (= darker part), the band being merged into a shading extending towards violet.

The deposit, which separated out of the solution acted on by the sodium amalgam, on the addition of hydrochloric acid was greenish, and dissolved in rectified spirit formed a bronze-green solution by daylight; by gaslight it appeared red in deep layers, and gave a spectrum like sp. 7, V. With ammonia it became a bright green (by daylight) and gave sp. 10, V.; the band at F had gone. These bands read:—1st about λ 656 to λ625 (?), 2nd λ 605 to λ 573. With ammonia and zinc chloride a faint red fluorescence was developed, the fluid being green, and showing a dark band in red, another feeble at D and a third nearer violet, reading:—1st λ 659 to λ 615, darker from λ 653 to λ 625, 2nd about λ 601 to λ 567·5, and 3rd in a thinner layer λ 514 to λ 496 (= darker part). With zinc chloride alone the colour became a darker green by daylight, and brownish by gaslight, with a greenish fluorescence and

the following bands appeared: 1st λ 656 to λ 619, 2nd λ 601 to λ 562 (?), 3rd in a thinner layer and in its darker part λ 517 to λ 499, shading off gradually towards violet. Sodium hydroxide developed similar bands only with slightly different readings, thus: 1st λ 656 to λ 625 ?, 2nd λ 605 to λ 571, and 3rd about λ 514 to λ 494.

The pigment (A) resembled (A) in the last experiment.

Further reduction of Hydrobilirubin.

There was a distinct difference between any of these colouring matters and any normal or pathological pigment discussed in the former part of this paper, and fearing that the supposed reduction had not been complete enough, I proceeded to carry it on further.

The action of the sodium amalgam aided by heat was therefore carried out until the fluid had become a pale yellow colour, the only band then visible in the solution being the one nearest violet[1] produced by the action of sodium hydroxide on hydrobilirubin. The solution was then acidified with hydrochloric acid, when it became a fine red colour; (but even before the acid was added it had begun to darken). It then showed a band covering D and a black one at F. It was then agitated with chloroform in a separating funnel when the chloroform became red. The latter was evaporated down and left a brown-red residue, which had a peculiar smell reminding of freshly prepared urobilin.

The rectified spirit extract of this residue was reddish-brown by daylight, redder by gaslight; a moderately deep layer showed a band covering D (Sp. 11, V.), and this extended from λ 605 to λ 573; the other dark band at F in a more dilute solution read from λ 501 to λ 475 in its darker part, although it gradually shaded off to λ 467.

Ammonia made the colour lighter and developed a feeble band in red and one at D, which read approximately: 1st λ 647 to λ 630 and 2nd λ 610 to λ 569, that at F had disappeared.

Ammonia and zinc chloride made the colour of the solution darker, and it now showed a green fluorescence and gave sp. 12, V. the bands reading: 1st λ 647 to λ 612·5, darker from λ 636 to λ 619, 2nd λ 599 to λ 566, 3rd in a thinner layer λ 517 to λ 494, with a shading getting gradually lighter up to λ 473. The colour of this solution when diluted was yellow; it had a rose tint in a more concentrated solution.

Zinc chloride alone made the colour of a spirit solution a lighter red and developed a green fluorescence, and bands very similar to those just

[1] In a very deep layer a shading was seen from λ 636 to λ 610.

described were seen: 1st λ 647 to λ 615, 2nd λ 601 to λ 566, 3rd in a thinner layer λ 514 to λ 496, in its darkest part, while the whole band in a more concentrated solution read from λ 520 up to λ 467, being gradually shaded off towards violet.

Sodium hydroxide produced bands very like the last: 1st λ 644 to λ 615, dark from λ 641 to λ 625, 2nd λ 599 to λ 562, dark from λ 593 to λ 573, 3rd in a thinner layer λ 514 to λ 496 in its darker part.

It is evident that as far as the spectra are concerned very little difference exists between the fully changed, and the less fully changed, pigment. There are probably bye-products formed during the action of the amalgam as I have said before and as Disqué points out. One of these is identical or closely related to the body which I am about to describe and which is found in bile. I formerly[1] observed, "It would therefore appear, that in addition to a body more closely resembling febrile (pathological) urobilin, which the fluid contained, an insoluble body was separated which appears to be identical with that kind of urobilin which occurs in bile and in gall-stones." And that is the opinion to which I am now led again by the present experiments. We are justified too in concluding that hydrobilirubin represents an intermediate stage of oxidation of bile pigment as I also formerly maintained, if indeed we are justified in concluding that in Gmelin's reaction the change is due to oxidation. I am well aware that hydrobilirubin bears a most remarkable and striking likeness to pathological urobilin and to stercobilin, but then there is the fact which cannot be got over that the bands which occur in its solutions on the addition of the reagents mentioned above do not occur in the case of solutions of these pigments. But I shall have more to say about this point further on.

On the Urobilin-like substance in Bile from the Gall-bladder.

I showed in the paper quoted before[2] that a substance which appeared to be urobilin exists in human, pig, ox, and sheep bile[3]. I have repeated these experiments recently and can confirm the former results, but owing to the importance which I now know must be attached to the bands in the red half of the spectrum, I think it necessary to describe

[1] *Proc. Roy. Soc. loc. cit.*

[2] *Proc. Roy. Soc.* No. 208, 1880.

[3] In the bile of the frog and toad I have noticed that caustic alkalis produce a band or bands like those of biliary urobilin treated in solution with the same reagents, the bile giving a red fluorescence under their influence.

these spectra and at the same time show wherein this colouring matter differs from others described here.

The bile was procured from the human gall-bladder after death; in the first experiment from a case of syphilitic tumour of the brain. The bile was treated with absolute alcohol and acetic acid and filtered. Owing to the strong absorptive power of the bilirubin for the violet end of the spectrum no band at F could be seen in the bile itself, but in the filtrate (after the above treatment) a band was seen before D from about λ 630 to λ 593. The extract was now diluted with water and agitated with chloroform, which became orange, and in very thin layers showed a distinct band at F. It was then evaporated on the water-bath and left a dark resinous-looking mass, in part green, in part dark and light yellow. It was then extracted with rectified spirit and the extract filtered; the resulting solution had a bronze colour with transmitted gaslight, and gave the band before D mentioned above in a deepish layer, while in a shallow one a band at F from λ 501 to 479, became detached; this band became much darker on adding hydrochloric acid. The band at D read approximately λ 625 to λ 581. On adding ammonia no band appeared in red, only one at D now reading λ 615 to λ 577? With ammonia and zinc chloride the colour became after a short time a fine green, and showed a red fluorescence, and then the bands appeared as shown in sp. 13, V which read: 1st λ 665 to λ 619, and dark part λ 659 to λ 630, 2nd λ 607·5 to λ 571, while in a shallower layer a band like that seen in a similar solution of hydrobilirubin appeared, reading from λ 514 to λ 496.

Zinc chloride alone produced very little effect, the colour remained as before, no new band appeared, but a broad ill-defined shading was seen at D, extending from about λ 625 to λ 569 and being probably composed of two bands, that at F remaining unchanged. This reaction however distinguishes this colouring matter from hydrobilirubin or any of the bye-products produced by the action of sodium amalgam on bilirubin as described above.

Sodium hydroxide changed the colour to orange red by gas-light, and then a band appeared in red, another at D and one nearer violet; these read: 1st λ 665 to λ 619, dark from λ 659 to λ 630, 2nd about λ 605 to λ 573, and 3rd λ 517 to λ 496. This solution soon changed to a dirty turbid green and showed then a red fluorescence.

In the next experiment the bile was removed from the gall-bladder of a patient who died from the effects of mitral disease. It was precipitated with absolute alcohol and filtered, but subsequently acetic acid

was added and it was again filtered. It was then diluted with water and agitated with chloroform, and the latter evaporated down, when it left a dark-brown resinous-looking residue. This was extracted with rectified spirit; the solution had a dull brown colour by reflected gas-light, deep red by transmitted, and it showed a band at D[1], as in the former case from about λ 625 to λ 581, where it shaded off gradually up to λ 552. In dilute solution the colour was yellowish green and a dark band was seen at F, beginning at λ 506, dark at λ 501 up to about λ 479. With ammonia the colour changed to a bronze green by daylight, yellowish-green by gaslight, and the solution showed a red fluorescence. This solution gave sp. 14, V; in a thinner layer, than that from which this spectrum was mapped the band at F had disappeared. These bands read approximately: 1st λ 662 to λ 625, dark from λ 653 to λ 636, 2nd about λ 605 to λ 573. With zinc chloride and ammonia the fluid appeared dull green with a red fluorescence, and showed the following bands: 1st λ 665 to λ 625 and dark from λ 653 to λ 636, 2nd a shading from about λ 605 to λ 573. In a thinner layer the band nearest violet read from λ 514 to λ 496, in its dark part. (sp. 15. V.) With zinc chloride alone a band was seen at D. This began to be shaded at λ 625 and ended about λ 552, and two bands placed closely together near F appeared, of which the first read λ 515·5 to λ 504 and the second λ 500 to λ 481. (See sp. 16, V., in which only part of the shading of 1st band is seen.) With sodium hydroxide a kind of red fluorescence was seen and these bands became developed, viz.: 1st 665 to λ 630, 2nd a shading λ 615 to λ 577, 3rd a shading 566 to λ 538 and 4th in a thinner layer, λ 517 to λ 496. The last became much darker after some time had elapsed.

Oxidation[2] *of this urobilin-like pigment with potassium permanganate.* The above rectified spirit extract after remaining exposed to the air for some time showed a shading after D as well as the band before it, and to this I added a weak solution of permanganate of potassium until these bands had gone. It was then agitated with chloroform which assumed a yellow colour, the chloroform solution was filtered; the filtrate was yellow and gave a band at F from λ 505 to λ 479 (=dark part, whole band from λ 511 to λ 473). On evaporating this it left a dark ochre-

[1] This disappears with ammonium sulphide, and becomes darker with peroxide of hydrogen, which also developes a band after D and one at F, showing that the band before D is due to oxidation.

[2] Probably by further oxidation choletelin would have been obtained, but I intend to try this again.

coloured residue partially soluble in ether, showing an intensely black band at F; it was partially soluble in chloroform, forming a yellow solution with a black band at F, insoluble in bisulphide of carbon and in benzene, slightly soluble in water and soluble in rectified spirit. The rectified spirit solution was orange (by gaslight): a deep layer transmitted red and a little green, and in thinner a black band at F, shading off towards violet. In concentrated solution the latter read from λ 506 to beyond λ 473, in a more diluted solution it read from λ 504 to λ 479, (See sp. 17 V.).

With ammonia the colour of an alcohol solution became orange-yellow and some feeble shadings in the red half of the spectrum were seen, that at F having disappeared. It was nearly impossible to read these, so that the following are only approximate measurements: 1st λ 659 to λ 630, 2nd a mere shading λ 605 to λ 573, 3rd λ 562 to λ 538.

With zinc chloride and ammonia a green fluorescence was developed and sp. 18, V., was seen, but the bands were too feeble to be read; that at F however in its darkest part extended from λ 515·5 to λ 496, being gradually shaded off towards violet. With zinc chloride alone the alcohol solution showed a faint green fluorescence; no bands could with certainty be detected in the red half of the spectrum, but in a dilute solution that at F was seen to be replaced by a double band: 1st from λ 517 to λ 504 and 2nd λ 501 to λ 477 (being darker than the first).

With sodium hydroxide the solution was pale yellow and the only band with certainty seen was one from λ 517 to λ 496.

Hence by oxidation the urobilin-like pigment of bile was changed into one more closely resembling pathological urobilin and hydrobilirubin, and I subsequently found that by mere exposure to the air the same result took place, for whereas when freshly prepared it showed with zinc chloride and ammonia a red fluorescence, on standing this changed to a green fluorescence. This helps to prove (as I have said before) that hydrobilirubin must not be looked upon as a pure reduction product, but as a product of intermediate oxidation; probably the same remark applies to pathological urobilin when of biliary origin as I have always maintained[1]. By further oxidation "biliary urobilin" can be made to resemble more closely choletelin and normal urobilin. In fact, the present investigation confirms at every step the conclusions which I was led to form as to the connexion between the colouring matters of blood, bile, and urine, in my former papers.

[1] It may be stated here that it is quite possible that in many cases pathological urobilin may be derived from two distinct sources, namely from bile and blood-pigments.

Summary and Remarks.

In the following table I have collected the most important measurements of bands produced in the various solutions by the reagents referred to before, and this table at a glance shows the connexion of, and the differences between, the various pigments. In some cases only the measurements of the darkest parts of the bands are given, and in some cases a band was left out when it was unimportant, as in the case of artificially prepared urohaematoporphyrin[1].

In the case of the alcohol solutions the constancy of the measurements of the band at F is very striking.

One can see at a glance also the following points, (1) the identity of normal urobilin with the product produced by the action of hydrogen peroxide on acid haematin; (2) the great similarity between pathological urobilin and stercobilin; (3) the probable origin of these from haematin at least in part, as many of the bands produced by ammonia, by that and zinc chloride, and by sodium hydroxide correspond to bands in the same solutions of urohaematoporphyrin and the same artificially prepared; (4) the striking difference between hydrobilirubin and any of these; (5) the difference between it and the urobilin-like substance of bile, and at the same time the resemblances between them; (6) the identity in all the solutions of the band nearest violet produced in them by the action of ammonia and zinc chloride, and by that of sodium hydroxide; (7) the closer resemblance of stercobilin to urohaematoporphyrin than to pathological urobilin; (8) the fact that some bands are missing from solutions of pathological urobilin and stercobilin when compared with similar solutions of urohaematoporphyrin: suggesting that the two former are more complete metabolites of haematin than the latter; (9) the points of resemblance between normal and pathological urobilin. Other interesting points also appear on a careful study of the table which need no mention here. With greater care and by the use of solutions of the same strength the resemblances would have come out even better marked.

It is difficult to say what influence a mixture of haematin and bile pigment-derivatives may have on some of the feeble bands, and doubtless such a mixture is present in some of these solutions.

[1] In the measurement of the spectra of the reduction products of haematin sometimes the 2nd, sometimes the 3rd product is taken, and some of the other measurements are taken from my note-book.

TABLE SHOWING IN WAVE-LENGTHS THE CONNEXION BETWEEN THE PIGMENTS REFERRED TO IN THE PAPER.

	Normal Urobilin	Haematin with H_2O_2	Pathological Urobilin	Stercobilin	Urohaematoporphyrin	Haematin $+Zn+H_2SO_4$	Haematin +Sod. Amalg.	Hydrobilirubin fully changed	Biliary Urobilin	Hydrobilirubin partially changed
Alcohol solution	504–479	504–479	607·5–587 577–552 504–477	607·5–577 504–479	630–619 607·5–599 581–555 588–520 506–481	625–615 583–560·5 540–523 504–477	633–619 610–599 585–550·5 544–521·5 506–479	605–573 501–475	625–581 501–479	610–549 504–477
Alcohol solution $+NH_4HO$	No band	No band	625–615 581–560·5 542–521·5 No band	636–619 587–562 549–526 517–494	625–615 581–562 540–523 511–494	625–612·5 583–562 540–526 514–490	633–617 587–562 547–529 514–490	647–630 610–569 Band absent	615–577 Band absent	Uncertain Band absent
Alcohol solution $+NH_4HO+ZnCl_2$	650–625 587–571 514–496	650–625 Shading 517–496	653–622 587–567·5 517–494	656–622 593–569 555–532 514–496	587–569 547–529 514–499	653–638·5 625–612·5 585–562 517–494	630–615 589–562 549–524·5 514–490	647–612·5 599–566 517–494	665–619 607·5–571 514–496	653–607·5 601–560·5 517–494
Alcohol solution $+NaHO$	653–625 514–496	647–625 514–496	653–625 597–577 571–559 517–496	653–625 593–569 549–529 520–499	625–615 581–562 542–526 515·5–496	625–612·5 585–560·5 588–529 514–496	See action of NH_4HO	644–615 599–562 514–496	665–619 605–573 517–496	650–617 601–566 517–494
Alcohol solution $+ZnCl_2$		517–494	583–573 517–496	591–569 559–532 515·5–499	585–569 547–529 514–499	625–607·5 585–566 549–532 517–494	633–615 585–562 547–526 514–488*	647–615 601–566 514–496	625–569 Unchanged	647–617 599–559 520–494

* From 2nd reduction product.

How can one account for the fact that stercobilin shows a relationship to urohaematoporphyrin and yet must certainly be in part derived from bilirubin? Probably the reply is that it is also in part derived from the haemoglobin of the food, being produced by the action of the digestive and putrefactive ferments on it. Doubtless the haemoglobin, or its derivative haematin, undergoes under the influence of these ferments the same changes which it undergoes out of the body by the action of such reagents as zinc and sulphuric acid, sodium amalgam &c.; being changed into haematoporphyrin and finally into stercobilin, the bilirubin of the bile sharing in the process. The haematoporphyrin, perhaps the haematin, and the changed bilirubin, being again taken up and carried by means of Schiff's circulation into the liver, and in this way accounting for the presence of the urobilin-like substance in bile.

The old theory that hydrobilirubin is produced in the intestine by the action of nascent hydrogen on bilirubin, and is then taken up into the circulation and excreted as urobilin in the urine, cannot be any longer maintained, because as I have shown, neither normal nor pathological urobilin is identical with that pigment. Doubtless in cases of recovery from jaundice we meet with changed bile-pigments in the urine which bear a resemblance to it, but even then the pigment formed in the urine is probably biliary urobilin or a metabolite of it.

If the haematoporphyrin and the bilirubin are again taken up into the liver from the intestine it is not impossible that they may be synthetized there (or elsewhere) into more complex substances. Nencki and Sieber[1] have found that bilirubin and haematoporphyrin have the same empirical formula, and that when introduced into the living subject haematoporphyrin is partly expelled in the urine, but the greater portion is retained and probably utilised in the formation of haemoglobin. But all the facts at present in our possession are not sufficient to allow of any theorizing in this direction. We know however that the amount of stercobilin expelled from the alimentary canal is not enough to account for all the bilirubin poured into the small intestine through the *ductus communis choledochus*.

The urobilin of normal urine is with difficulty imitated by the artificial production of a similar substance—choletelin—from bile pigment, while it can be produced with great ease from haematin; hence probably it is produced normally by the action of nascent oxygen in the tissues on haematin. If this be so how can we account for its

[1] *Monatsh.* IX. 115—132.

being present as a chromogen, a reduction product in urine? Easily enough, as I stated at the beginning of this paper, as the urine is now known to contain reducing substances which probably exert their action in the bladder or perhaps even in the kidney. But why should we find —as I formerly found—evidence of the presence of a second chromogen in urine which sometimes resembles that of pathological urobilin? If we imagine some stercobilin to be taken up from the intestinal canal this is easily explained, as it too would become reduced in the urine. Or since, by further reduction, normal urobilin appears capable of being converted into pathological urobilin, we have only to suppose that a part of the latter becomes more reduced than another part.

In face of the facts brought to light by the observations described in this paper, the following would be the simplest way of explaining the origin of all the colouring matters referred to. I do not wish this explanation to be accepted as final, I merely suggest it until something better takes its place.

Bilirubin and biliverdin are produced in the liver mainly from effete haemoglobin[1]; these are acted on in the small intestine by the digestive and putrefactive ferments, and some at least changed into simple metabolites like the urobilin-like substance of bile. The haemoglobin, and histohaematin of meat, or their metabolites, are by the influence of the same ferments acted upon in the same manner, and carried with the changed bile pigments through the branches of the portal vein into the liver, where they undergo changes of which we are at present ignorant. A portion of both the bile- and haematin- derivatives are however passed on along the intestinal canal and form stercobilin. This may under certain unknown conditions be taken up, probably accompanied by ptomaines, and excreted in the urine as pathological urobilin.

But sometimes we meet with pathological urobilin which shows such a resemblance to Le Nobel's urobilinoidin as to lead one to suppose that it is entirely derived from haematin, or sometimes it is accompanied, or replaced by, urohaematoporphyrin, the latter having undoubtedly no biliary origin; in such cases it must be produced by destruction of haemoglobin or histohaematin in various tissues besides the liver, the normal metabolism being increased to such an extent that its products are incapable of oxidation, and they therefore appear in the urine in the state of urobilinoidin or urohaematoporphyrin.

Normally, doubtless, the liver and other blood-metabolising glands

[1] I have shown elsewhere (*Philos. Trans.* Pt. II. 1885) that biliverdin occurs in *Actinia mesembryanthemum*, being there derived from a pigment which yields haemochromogen.

are able to pick the effete pigments out of the circulation and change them into bile pigments or others, and to a certain extent do so even when they are present in slight excess, but under certain conditions these organs are unable to deal with the excess of pigment. That this is so, is proved by the presence of urohaematoporphyrin in the urine in cases of cirrhosis of the liver and of Addison's disease, as I have shown[1]. There is very little doubt that in acute rheumatism a very large amount of urohaematoporphyrin is formed, and in that disease probably the muscles are the seat of the formation of a great part of it[2]. Indeed it would appear that in some cases the presence of urohaematoporphyrin indicates the existence of some very active fermentation attended by energetic reduction, but beyond this one can say very little else with our present knowledge.

I venture to give a rough diagram, Pl. XV, showing the connexion between the pigments referred to. Of course other complications might be introduced into the diagram, but at present they are best left out.

It remains to be seen how far these results will be confirmed by other chemical methods, but we must remember that in the case of animal colouring matters the spectroscopic has outstripped other methods of analysis, and shows resemblances and differences between the pigments which by such methods would escape detection.

The following five charts of spectra were drawn from measurements made with the microspectroscope, but owing to the small dispersion of that instrument I do not guarantee that they will agree with those given in the text. The latter were calculated by means of a one prism spectroscope made for me by Mr Adam Hilger, and were worked out by means of interpolation curves.

EXPLANATION OF CHARTS OF SPECTRA.

Plates.

Chart I. Plate X.

Sp. 1. Alcohol solution of pathological urobilin treated with ammonia. The spectrum represents the result of examining three different depths of solution.

Sp. 2. Alcohol solution of pathological urobilin treated with zinc chloride and ammonia: deep layer.

[1] This *Journal*, Vol. VI. Nos. 1 and 2, and *Brit. Med. Journ.* Feb. 4, 1888.

[2] Myohaematin being probably its source, for the histohaematins as I have shown can be made to yield haematoporphyrin. This *Journal*, Vol. VIII. No. 2.

Sp. 3. The same, shallower layer.

Sp. 4. Influence of zinc chloride alone on an alcohol solution of the same urobilin: a combination spectrum mapped from a deep and shallow layer.

Sp. 5. Influence of sodium hydroxide on an alcohol solution: a combination spectrum of a deep and shallow layer.

Sp. 6. Acidulated alcohol extract from lead precipitate from urine of case described in the text. Bands of acid urohaematoporphyrin.

Sp. 7. Alcohol solution of the isolated pigment, from same urine treated with ammonia.

Sp. 8. Alcohol solution of the same treated with ammonia and zinc chloride.

Sp. 9. Alcohol solution of the same treated with zinc chloride only.

Sp. 10. Alcohol solution of urohaematoporphyrin from case of measles. (Combination spectrum of deep and shallow layer.)

Sp. 11. The same solution treated with ammonia (also a combination spectrum).

Sp. 12. Influence of ammonia and zinc chloride on an alcohol solution of the same (after several hours' action).

Sp. 13. Influence of zinc chloride alone on an alcohol solution; note band at F has not shifted.

Sp. 14. Alcohol solution of isolated pigment from urine of case of meningitis; it presents some of the characters of pathological urobilin and of those of urohaematoporphyrin. A combination spectrum mapped from three different layers of fluid.

Sp. 15. Influence of ammonia[1] on the same solution.

Sp. 16. Influence of ammonia and zinc chloride on the same, the band at D is rather too dark.

Sp. 17. Influence of zinc chloride alone on an alcohol solution (see text). This map is drawn from three different depths of solution, and the bands between D and E are therefore somewhat darker than they appeared.

Sp. 18. Deep layer of alcohol solution treated with sodium hydroxide.

Sp. 19. Action of ammonia on an alcohol solution of the pigment isolated from case of typhoid fever.

Sp. 20. Action of ammonia and zinc chloride on the same. Combination spectrum of a deep and shallow layer.

Chart II. Plate XI.

Sp. 1. Pigment from urine of case of intra-peritoneal haemorrhage, isolated by the lead method and dissolved in rectified spirit.

Sp. 2. Influence of ammonia on the same solution.

[1] Probably the feeble narrow bands in red are not correct in this spectrum, cf. measurements in the text.

Sp. 3. The same solution with zinc chloride and ammonia. A combination spectrum from a deep and shallow layer.

Sp. 4. Pigment from urine of case of haemorrhage into leg, isolated and acted on with ammonia in alcohol solution.

Sp. 5. Action of ammonia and zinc chloride on an alcohol solution of the same pigment.

Sp. 6. Pigment from urine of case of peritonitis; isolated and dissolved in rectified spirit: a combination spectrum of a deep and shallow layer.

Sp. 7. The same solution treated with ammonia. (A combination spectrum from a deep and shallow layer.)

Sp. 8. Action of zinc chloride and ammonia on an alcohol solution of same (combination spectrum).

Sp. 9. Haematoporphyrin dissolved in rectified spirit and sulphuric acid (= acid haematoporphyrin).

Sp. 10. Chloroform solution from this.

Sp. 11. Isolated haematoporphyrin in rectified spirit: showing a five-banded spectrum.

Sp. 12. Four-banded alkaline haematoporphyrin, got by dissolving haematoporphyrin in alcohol and ammonia.

Sp. 13. Action of zinc and sulphuric acid on haematin, early stage of reduction, the band at F indicates that it has gone beyond the haematoporphyrin stage.

Sp. 14. Chloroform solution from the last, note the dark band nearest violet, and yet the pigment has not reached the urohaematoporphyrin stage. (This is a combination spectrum.)

Sp. 15. Rectified spirit solution of the first reduction-product from acid haematin (produced by Zn and H_2SO_4.)

Sp. 16. Action of ammonia on the last solution. Showing a spectrum like, but not yet identical with, alkaline urohaematoporphyrin.

Sp. 17. Further action of zinc and sulphuric acid on haematin: the spectrum of the acidulated alcohol solution. (A combination spectrum of a deep and shallow layer cf. sp. 9.)

Sp. 18. Chloroform solution from the last. (A combination spectrum.)

Sp. 19. Rectified spirit solution of the isolated residue from the last solution. (A combination spectrum.)

Sp. 20. Action of ammonia on this solution.

Sp. 21. Action of zinc chloride only on the solution whose spectrum is shown in sp. 19.

CHART III. PLATE XII.

Sp. 1. Further reduction-product from haematin by the action of zinc and sulphuric acid: acid alcohol solution. (Combination spectrum.)

Sp. 2. Rectified spirit solution of the isolated pigment. (Combination spectrum.)

Sp. 3. Action of ammonia on this solution. (Combination spectrum.) The 5th band is rather too dark.

Sp. 4. Action of ammonia and zinc chloride on the same solution, shallow layer, the bands of a deep layer resemble those of sp. 3, in all points except the band nearest violet which has the peculiarity of shading of the urobilin (pathological) band.

Sp. 5. Action of zinc chloride alone on an alcohol solution of the same colouring matter.

Sp. 6. Action of sodium amalgam on haematin, early stage of reaction, rectified spirit (diluted) solution.

Sp. 7. The same acidified with sulphuric acid, showing that the solution has not gone much beyond the haematoporphyrin stage, although a band has appeared at F. (The two first bands are rather too near the red end of the spectrum.)

Sp. 8. Chloroform solution from the last.

Sp. 9. Rectified spirit solution of the pigment isolated from the last solution.

Sp. 10. Action of ammonia on the last solution.

Sp. 11. Action of zinc chloride alone on a rectified spirit solution of the same pigment. Note that the band nearest violet has not shifted. The third band from the red end is shown rather too dark.

Sp. 12. Further reduction product in rectified spirit. (Containing sodium hydroxide.)

Sp. 13. The same solution acidified with sulphuric acid.

Sp. 14. Rectified spirit solution of the isolated pigment.

Sp. 15. The third reduction product got by the action of sodium amalgam on haematin: in rectified spirit (containing sodium hydroxide): before its isolation.

Sp. 16. Chloroform extract from the last solution.

Sp. 17. Rectified spirit solution of the isolated pigment. (A combination spectrum.)

Sp. 18. Action of ammonia on the last solution; the spectrum will also serve to show the action of ammonia and zinc chloride on the same.

Chart IV. Plate XIII.

Sp. 1. Acidulated alcohol extract from the precipitate produced in normal urine by neutral and basic acetate of lead. Note absence of bands in red half of spectrum.

Sp. 2. The same solution; thinner layer, showing the band of normal urobilin.

Sp. 3. Absolute alcohol solution of the isolated pigment, thin layer.

Sp. 4. Action of ammonia on a deep layer of the same.

Sp. 5. Action of ammonia and zinc chloride on an absolute alcohol solution of urobilin. (Combination spectrum.)

Sp. 6. Normal urobilin (from another specimen) in alcohol solution with zinc chloride only. Note band at F (which is here broader than usual).

Sp. 7. Artificially prepared normal urobilin, got by action of peroxide of hydrogen and subsequently sodium amalgam on haematin: rectified spirit solution of the isolated colouring-matter.

Sp. 8. Action of ammonia and zinc chloride on the last solution. (Combination spectrum.)

Sp. 9. Stercobilin (isolated as described in the text) dissolved in rectified spirit. (Combination spectrum.)

Sp. 10. Action of ammonia on the last solution.

Sp. 11. Action of ammonia and zinc chloride on a similar solution. (Combination spectrum.)

Sp. 12. Action of zinc chloride alone on an alcohol solution. (Combination spectrum.)

Sp. 13. Acidulated alcohol extract of stercobilin from another source. A combination spectrum from three different thicknesses of fluid.

Sp. 14. Action of zinc chloride only, on a rectified spirit solution of the isolated stercobilin. (Combination spectrum.)

Sp. 15. Action of sodium hydroxide on a rectified spirit solution. (Combination spectrum.)

Sp. 16. Filtrate from a solution of bilirubin in dilute caustic soda solution, to which some alcohol had been added; after incomplete action of sodium amalgam.

Sp. 17. Chloroform solution from this, got by acidulating, and then agitating with chloroform.

Sp. 18. Isolated colouring matter from the last solution in absolute alcohol. (Combination spectrum.)

Sp. 19. Absolute alcohol solution of the same treated with ammonia.

Sp. 20. Action of ammonia and zinc chloride on an alcohol solution. (Combination spectrum.)

Chart V. Plate XIV.

Sp. 1. Action of zinc chloride alone on an alcohol solution of the above intermediate product. (Combination spectrum.)

Sp. 2. Action of sodium hydroxide on a similar solution.

Sp. 3. The precipitate (B, see text) which formed, when the partially reduced solution of bilirubin having been treated with hydrochloric acid, was filtered off, dissolved in alcohol, diluted with water, hydrochloric acid added,

and then agitated with chloroform, the chloroform evaporated, the residue dissolved in alcohol and treated with zinc chloride and ammonia.

Sp. 4. An alcohol solution of the same pigment treated with zinc chloride alone.

Sp. 5. Solution of bilirubin in aqueous soda treated with sodium amalgam (in another experiment).

Sp. 6. The last solution was acidulated with hydrochloric acid, filtered, and the filtrate agitated with chloroform, which gave this spectrum.

Sp. 7. Residue (C) from the chloroform extract dissolved in rectified spirit. (Combination spectrum.)

Sp. 8. Action of ammonia and zinc chloride on such a rectified spirit solution (deep layer).

Sp. 9. The same solution (thinner layer).

Sp. 10. A deposit (B) produced by adding hydrochloric acid to the sodium-amalgam-treated-fluid, was separated by filtering, dissolved in rectified spirit, and gave this spectrum on adding ammonia.

Sp. 11. Complete action of sodium amalgam on bilirubin. Isolated colouring matter (i.e. hydrobilirubin) dissolved in rectified spirit. (Combination spectrum.)

Sp. 12. Action of ammonia and zinc chloride on a rectified spirit solution of the same hydrobilirubin. (Combination spectrum.)

Sp. 13. Action of zinc chloride and ammonia on a rectified spirit solution of the urobilin-like pigment of human bile. (Combination spectrum.)

Sp. 14. Action of ammonia on a rectified spirit solution of "biliary urobilin."

Sp. 15. Action of ammonia and zinc chloride on a similar solution. (Combination spectrum.)

Sp. 16. Action of zinc chloride alone on a rectified spirit solution of the same colouring-matter. (Combination spectrum.)

Sp. 17. Rectified spirit solution of "biliary urobilin" after this pigment had been oxidized by potassium permanganate: showing disappearance of all the bands except that at F.

Sp. 18. Action of ammonia and zinc chloride on the last solution: this showed a green fluorescence instead of a red one, showing that by oxidation "biliary urobilin" is made to resemble hydrobilirubin or pathological urobilin, and proving that the "biliary urobilin" is probably an intermediate product of oxidation.

Owing to the shortness of these spectra some slight errors of position may have crept in, but these can be corrected by the wave-length measurements given in the paper.

PLATE XV.

Scheme showing the probable derivation of urobilin, &c., from haemoglobin.

ON THE NATURE OF THE KNEE-JERK. By WARREN PLIMPTON LOMBARD, M.D., *Assistant in Physiology at the College of Physicians and Surgeons of New York.*

It has long been known that if the ligamentum patellae of a normal individual be struck when the leg is hanging freely in a position of partial flexion, the quadriceps muscle is irritated and contracts, causing a sudden extension of the knee, which we call a knee-jerk. The process by which the muscle is stimulated, however, is still a subject of discussion.

Some authors claim that the muscle fibres are directly stimulated by the sudden twitch to which they are subjected, the method of excitation being similar to that resulting from a direct blow on the muscle, and these writers think that the time which elapses between the moment of the blow on the ligament and the resulting muscular contraction is far too short to admit of any other interpretation.

Other authors call attention to the fact that the phenomenon cannot be obtained if the nerve connecting the muscle with the spinal cord first be severed, or if the corresponding reflex mechanisms of the cord have been injured by disease, and conclude that since the integrity of the reflex arc is essential to the phenomenon the knee-jerk must be a reflex act. They admit that the time involved in the process is very short, but say that the time required by such a reflex action, as they suppose this to be, is not known, and that it may well be as short as the interval found.

Those who regard the process to be wholly peripheral, answer the objection that it is lost if the reflex arc be destroyed, by suggesting that the ability of a muscle to respond to such a slight mechanical stimulus as that caused by a blow on its tendon may depend upon its having a certain amount of tension, and that this necessary tension is dependent on the tonus of the muscle, which is lost, when, upon division of the nerve or destruction of the cord, tonus impulses cease to come to the muscle.

Thus we find two distinct parties; the one regards the phenome-

non reflex, the other considers it a wholly peripheral process. Both admit that the integrity of the reflex arc is essential to the knee-jerk, and that the time involved in the process is much shorter than that required by the few reflex acts of which the time has been studied.

Undoubtedly the reflex theory offers the simplest and most satisfactory explanation of the intimate dependence of the phenomenon upon the spinal cord. Indeed the time argument would have but little weight if those who doubt the reflex nature of the process were unable to explain this connection. It is worth while, therefore, to consider the soundness of their explanation.

As has been said, they assume that the ability of the muscle to respond to the twitch transmitted to it from its ligament is dependent on the tension of the muscle, and that this is dependent on tonus impulses coming to it from the spinal cord.

One cannot help feeling that this explanation has been manufactured to meet the emergency and is somewhat forced, both because of the uncertain basis on which the tonus theory rests, and because there is no proof that the irritability of a muscle to mechanical stimuli is dependent on its tension.

The knee-jerk, though it varies greatly in amount, can almost always be obtained, while there is reason to doubt that muscles are kept continuously in tonus by impulses either of automatic or of reflex origin. It is most probable that there are times when they receive slight motor impulses of reflex nature, and that they respond by the feeble increase in tension which we call tonus, but there is no reason to suppose that they are continually in receipt of tonus impulses.

Suppose, however, for the sake of argument, that tonus is continuous, there is no surety that it is essential to the knee-jerk. It is true that for purely mechanical reasons, tension of the muscle not only favors but is necessary to the phenomenon. If the blow be struck when the ligament and muscle are loose, there is no twitch transmitted to the muscle and consequently no knee-jerk developed. On the other hand, if the slack has been taken up, the sudden strain caused by the blow on the ligament is transmitted through the whole muscle. Moreover, when the muscle is under tension the smallest contraction will reveal itself. By giving the leg a proper position, one can secure to the muscle any desired degree of tension, this, however, does not enable one to get a knee-jerk when the spinal cord is diseased.

If tonus be essential to the phenomenon it would seem to be for some other reason than because it supplies a required tension. One

might conceive that in some unknown way it increases the irritability of muscle tissue. If this be true, however, it would seem as if the knee-jerk which disappears when the tonus of the muscle is lost, upon division of its nerve, would be obtained again, when, by electrical stimulation of the cut-nerve, the tension of the muscle is restored. This experiment has been made upon a rabbit but failed to recall the knee-jerk.

This last winter I examined a man who was young, vigorous and showed no sign of disease. His legs were well developed, and his muscles firm and elastic. There was no sign of lack of tension of the muscles. He claimed to be the equal of his fellows in all manly sports. I could not prove that he had or had not tonus of the muscles, but I saw no reason to doubt that he had as much as most men. This man gave no knee-jerk and no reenforcement of the knee-jerk.

It is not uncommon to find a person in good health with no knee-jerk, though it is exceptional to find one from whom a reenforced knee-jerk cannot be obtained. If the absence of the knee-jerk in these cases be due to a lack of tonus, and if muscle tonus plays the important rôle attributed to it, it is strange that these men should show no disturbance of muscular co-ordination. Indeed, such cases tend to show that something more than a suitable condition of the muscles is essential to the production of the knee-jerk.

Any theory of the nature of the knee-jerk, to be acceptable, must likewise explain the reenforcements of the knee-jerk. It occurred to me, therefore, that a study of the reenforcements of the knee-jerk might throw much light on the nature of the phenomenon. If the processes on which the knee-jerk depends are wholly peripheral, the increase of the contraction which results from the reenforcing act must necessarily be due to changes occurring within the peripheral mechanisms. How might the ability of a muscle to respond to a blow on its tendon be increased by a reenforcing action? Two ways suggest themselves, viz.:—

1. The tonus of the muscle might be increased as a result of an increased activity of the spinal centres, which might be caused by the development of a voluntary action, a painful sensation or an active emotion or thought.

2. The irritability of the muscle might be increased in some inscrutable way by the reenforcing process.

I made a careful study of the quadriceps muscle and tried to discover whether it gained in tension or in irritability when the hands were

clinched. I found it difficult to construct an apparatus capable of measuring delicate changes in muscle tension, but finally fixed upon one which answered the requirements fairly well. It recorded the smallest contraction of the quadriceps which I could learn to make voluntarily, and this contraction was so slight that it could not be detected by the eye or by a trained hand placed over the muscle. It seemed as if a smaller increase in tension than this could scarcely be regarded as sufficient to influence the ability of the muscle to respond to the blow, either because the tenser muscle fibres would favor the transmission of vibrations, or because, the slack having been taken up, the muscular contraction would reveal itself more readily.

The apparatus used to record the contraction of the quadriceps consisted of a long, light, stiff lever, the axis of which was fastened by an elastic band upon the ligamentum patellae. The lever rested, near its axis, on the lower edge of the patella. When the quadriceps contracted it made the ligament more tense, and at the same time tilted the patella; both of these movements caused the recording end of the lever to descend. The lever magnified the movement of the ligament and patella about twenty times.

The man lay on his back, on a bench made with adjustable segments, which supported the head, back, thighs, legs and feet in any desired degree of flexion. A control lever, which recorded every movement of the head of the tibia, wrote the movements of the knee caused by the respiration, the pulse and jars.

A Marey's tambour, connected with a dynamometer, wrote with its lever the moment that the reenforcing movement of clinching the hands was performed.

Not to go further into details, suffice it to say that no contraction of the quadriceps was seen to follow the most active hand clinch in the case of any of the normal men experimented on, though such an action was very potent in reenforcing the knee-jerk.

There is one experiment which deserves separate mention. The young man in question had no knee-jerk, but showed an active reenforced knee-jerk. It seemed as if in his case, at least, one might find a change in the tension of the quadriceps, i.e. if the knee-jerk which appeared when he reenforced depended on an increase of tonus, but none was found.

From these experiments it would seem that such an act as clinching the hands, though capable of reenforcing the knee-jerk, affects the tension of the quadriceps muscle little, if at all, under normal conditions.

Finding no increase in the tonus of the muscle when the hands were

clinched, I proceeded to test the irritability of the muscle at the time that the reenforcing act was being performed. I used as a test, the strength of the induction current required to call out the least contraction which the lever already described was capable of recording. No increased irritability to electrical stimulation was discovered. It may be said that the irritability of the muscle to mechanical stimuli may, nevertheless, have been enhanced. This seems doubtful, however, for as a rule when the irritability of a muscle is increased, it responds more readily to all forms of stimuli.

In short, I was unable to find any evidence that, in the case of normal men, lying at rest, such an act as voluntarily clinching the hands has any effect on either the tension or the irritability of the muscle. This result corresponds with that of Mitchell and Lewis[1], who found that muscular contractions called out by electricity cannot be reenforced, a result which would be inexplicable in case the reenforcing influence affected the peripheral mechanisms.

If these results are correct, the knee-jerk cannot be a purely peripheral phenomenon and is, probably, in spite of the short time, a reflex process. This conclusion is borne out by the results of experiments which I made at the College of Physicians and Surgeons of New York, during the past winter.

These experiments were made on twenty-five medical students, all of whom were, as far as could be judged, in good health. It is worth mention that they were wholly unfamiliar with the work and that they had no idea of what was expected of them.

The apparatus was the same as that employed by me in the experiments reported in the *American Journal of Psychology*, Oct., 1887. It consisted of a couch, on which the subject lay on his left side; a support for the right thigh; a swing for the right foot, suspended by a long cord from the ceiling; a recording needle, which was attached to the right heel, and which by writing the movements of the foot gave a magnified record of the flexion and extension of the knee; and finally a hammer which delivered a blow of constant and known force.

A clear understanding of the results obtained is only to be reached by a careful analysis of the conditions of the experiment. When the subject is placed on the knee-jerk couch with his thigh on the

[1] "Physiological Studies of the Knee-jerk, and of the Reactions of Muscle under Mechanical and other Excitants," by S. Weir Mitchell, M.D., and Morris J. Lewis, M.D., *The Medical News of Philadelphia*, Feb. 13 and 20, 1886.

support and his foot in the swing, the knee assumes a position of partial flexion. If the experimentor draws the lower leg forward, so as to increase the extension of the knee, or backward, so as to increase the flexion of the knee, and then release it, the leg swings like a pendulum backwards and forwards for eight or ten times, each oscillation being smaller than the preceding, until the leg becomes quiet in the original position of rest. What are the forces which determine how much the knee shall be flexed when in the position of rest?

1. The force of gravity, which tends to bring the swing and foot directly under the point of suspension of the swing.

2. The amount of flexion of the hip joint and the consequent degree of tension of the two joint muscles on the front and back of the thigh.

3. The tension of the muscles on the front and back of the thigh dependent on their natural length and elasticity.

4. The contraction of the muscles caused by nervous or other stimuli.

It may seem a contradiction to speak of muscular contraction as one of the conditions which determine the position of the leg when at rest, but, inasmuch as the theory of muscle tonus still holds, it may not be inadmissible.

Most of the forces enumerated act constantly and continuously. If the body and thigh are immovable, the tension of the muscles of the thigh, as far as it is governed by the position of the hip joint, will be uniform; the elasticity of the muscles will undergo but little change during short intervals of time; the influence of the force of gravity will remain the same; therefore, any change of the knee from the position of rest, must be caused by a difference in the amount of contraction of the antagonistic flexors and extensors.

If these muscles were in receipt of tonus impulses which were continuous and constant, their length would undergo no change on this account; if, however, they received irregularly tonus impulses of varying amounts, their length would change frequently. I was curious to find whether such variations actually occurred, but in the few experiments which I made, I could not discover that the knee moved at all from the position of rest when the subject was calm and was lying quietly.

Now what happens when the ligamentum patellae is struck? The blow is immediately responded to by a contraction of the muscles on the front of the thigh, the knee is extended, and the foot is swung forward.

As the extensor muscles cease to contract, the elasticity of the flexor muscles together with the force of gravity cause the foot to swing back; the force of the backward swing is sufficient to carry the foot beyond the position of rest and to flex the knee, though not as greatly as the contraction of the muscles had extended it; this flexion of the knee is in turn followed by extension, and this by a second movement of flexion, and so on, each act being less than the preceding, until after eight or ten oscillations the knee becomes quiet in the position of rest.

Such is the ordinary sequence of events following a blow on the ligamentum patellae, and, when the subject is in a placid condition, the knee actually returns to the original position of rest time after time, as often as the experiments are repeated. This is illustrated by experiments on S, recorded in Table I., during which the knee invariably returned to the original position of rest.

Description of Table I.

The table is to be read from left to right. In the first column is the number of the observation; in the second, the extent of the knee-jerk or first movement of extension; in the third, the first backward oscillation of flexion; in the fourth, the second oscillation of extension; in the fifth, the second oscillation of flexion; while in the sixth column is recorded the change in the position of the line of rest from that which it occupied before the blow was struck. In this, as in all succeeding tables, the sign + signifies a movement in the direction of extension, recorded in the charts as an upward movement, while the sign – indicates a movement of flexion, recorded in the charts as a downward movement. The figures give the extent of the movement in millimetres. The measurements were all made from the line of rest which was drawn just before the blow was struck.

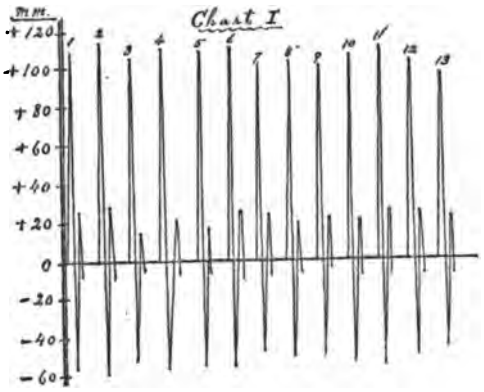
TABLE I.

S, Curve 31.

No. of Observation	1st Extension or Knee-jerk	1st Flexion −	2nd Extension +	2nd Flexion −	Change of position of line of Rest
1	110	55	26	7	0
2	113	58	28	9	0
3	105	52	15	5	0
4	111	56	21	7	0
5	109	55	18	6	0
6	111	55	27	9	0
7	102	49	24	7	0
8	103	50	20	6	0
9	101	50	23	6	0
10	106	53	21	7	0
11	110	55	25	8	0
12	103	51	24	8	0
13	96	46	21	7	0
14	103	51	21	8	0
15	105	52	20	8	0
16	84	43	18	7	0
17	83	42	20	7	0
18	92	45	22	8	0
19	103	52	25	9	0
20	96	48	25	8	0
21	95	47	24	8	0
22	84	39	19	7	0
23	88	43	21	7	0
24	99	50	26	9	0
25	92	45	23	7	0
26	95	48	25	8	0
27	92	46	22	8	0
28	99	51	27	9	0
29	106	53	30	10	0
30	108	55	30	10	0
31	111	56	31	10	0
32	98	46	22	6	0
Average	100	50	23	8	

The regularity of the oscillations is well shown in Chart I., which was constructed from the same curves as this table. The extent of the movements has been reduced three-fourths, which necessitated the omission of the final small oscillations.

S, Curve 31.



As has been said, it is usual for the knee to return to the original position of rest when the subject is in a tranquil frame of mind. When, however, the subject is excited, it is much more common to see the knee become quiet in a different position, and be either more extended or more flexed than before the blow was struck. This is illustrated by the measurements given in the following Table.

Description of Table II.

In this table are collected the changes in the position of the line of rest which occurred in experiments on seven different men. The observations are arranged in columns. At the head of each column is the letter which stands for the subject, and beneath this is the number of another table in this paper, in which the same results are given. Below this heading are recorded the successive changes in the position of the line of rest. The measurements are given in millimetres, and each was made with reference to the position of the line of rest at the end of the preceding observation. The sign + shows that the variation was in the direction of extension, and the sign – that it was in the direction of flexion.

TABLE II.

SUBJECT	A	G	X	D	C	L	U	C	A
No. of Table	I	V	V	V	V	VI	VI	VII	IX
Changes in the position of the line of Rest seen to follow the successive Knee-jerks — 1	0	0	0	+2	+2	+ 2	− 2	+ 5	− 4
2	0	0	0	+2	0	+ 1	− 3	− 1	− 5
3	0	0	0	+1	+1	− 6	+ 1	+11	+ 5
4	0	0	0	0	+2	− 5	+ 7	+ 2	− 5
5	0	0	0	+1	0	+ 1	+ 2	+ 7	+ 5
6	0	0	0	0	0	+ 6	− 7	− 5	− 7
7	0	0	0	0	0	+ 4	− 4	+ 2	+ 1
8	0	0	0	0	0	− 1	− 7	− 1	+ 8
9	0	0	0	0	−1	+ 1	+ 6	0	+11
10	0	0	0	0	+1	+ 6	+ 4	0	+ 7
11	0	0	0	0	0	− 1	+ 2	0	−18
12	0	0	0	0	+1	+ 1	− 1	+25	+ 7
13	0	0	0	0	0	− 1	+ 8	+ 5	− 1
14	0	0	0	0	−1	?	+15	+ 2	0
15	0	0	0	0	0	*	+ 6		+ 1
16	0	0	0	0	+1	+ 2	+ 4		?
17	0	0	0	0	0	+ 1	*		*
18	0	0	0	0	0	+ 2	− 3		− 5
19	0	0	0	0	0	+13	+ 3		− 8
20	0	0	0	0	0	+ 1			0
21	0	0	0	0	+1	− 9			− 3
22	0		0	0	0	+18			+ 5
23	0		0	0	0	− 1			+12
24	0		0	0					+ 2
25	0			0					− 5
26	0								

* Break in curve.

During the examinations which are recorded in the first five columns there was little or no change of the position of the line of rest, while during the examinations reported in the last four columns considerable variations occurred. It was noticed that in all the cases in which marked changes in the position of the line of rest appeared, the men were in an excitable condition. Almost all the men were slightly disturbed by the first examination. In the case of A and C the records which show the greatest variations were those obtained the first time they were examined, while the records which show no variations were gained at a subsequent examination.

It is evident then that the knee may return after each knee-jerk to the position held before the blow was struck, or that it may come to rest in a new position, which may be more flexed or more extended than before the knee-jerk was called out. As has been shown such changes in position can only result from an alteration of the tension of the flexor and extensor muscles of the knee, and that this was really the cause of the change of position will become still more evident when we study the relative size of the oscillations which followed the knee-jerk.

Omitting for the present the causes for the varying degrees of tension found, let us see what effect they exerted upon the extent of the knee-jerk. It is to be remembered that the fact that the spinal cord is essential to the development of the knee-jerk is explained by those who disbelieve that it is a reflex act, on the ground that the spinal cord is indispensable to the tonus of the muscle, and that the muscle must have a certain amount of tension in order to be able to respond to blows on its tendon. If it be true that the irritability of a muscle to mechanical stimuli is dependent on its tension, it would seem that the extent of the knee-jerk would vary with the degree of tension of the muscle. In the experiments recorded there is no lack of changes of tension of the muscle or of variations in the size of the knee-jerk, but the degree of tension and the size of the knee-jerk are entirely independent of one another. The truth of this statement is proved by the following table, in which the changes in the tension of the muscles as determined by the variations of the position of the line of rest are recorded side by side with the height of the knee-jerk which was called out immediately after this change of tension.

Description of Table III.

The table is headed by the letters which represent the names of the subjects examined, and beneath each of them is the number of another table of this paper in which these same results appear. Below each letter the results of the examination are arranged in two parallel columns. In the first is recorded in millimetres the variations in the position of the line of rest which occurred in the succeeding observations, the sign + indicating a movement towards extension and the sign – towards flexion. In the second column is stated the extent of the knee-jerks which were called out when the muscles had the tension recorded on the same line in the first column.

TABLE III.

A		C		L		U		U	
IX		VII		VI		VI		(curve 61)	
Line of rest	Knee-jerk	Line of rest	Knee-jerk	Line of rest	Knee-jerk	Line of rest	Knee-jerk	Line of rest	Knee-jerk
0	81	0	63	0	−102	0	81	0	55
− 4	70	+ 5	56	+ 2	59	− 2	60	− 4	48
− 5	50	− 1	60	+ 1	51	− 3	71	+10	51
+ 5	56	+11	61	− 6	68	+ 1	77	+ 7	66
− 5	53	+ 2	44	− 5	58	+ 7	85	+ 6	47
+ 5	48	+ 7	41	+ 1	73	+ 2	?	− 2	51
− 7	61	− 5	52	+ 6	81	− 7	55	+ 3	50
+ 1	63	+ 2	49	+ 4	72	− 4	65	− 4	51
+ 8	51	− 1	55	− 1	72	− 7	95	0	67
+11	88	0	70	+ 1	72	+ 6	94	+ 3	60
+ 7	30	0	45	+ 6	64	+ 4	75	− 1	61
−18	60	0	82	− 1	75	+ 2	96	− 5	67
+ 7	50	+25	36	+ 1	72	− 1	79	− 2	65
− 1	39	+ 5	24	− 1	66	+ 8	88	− 7	67
0	37			?	*	+15	81	− 7	65
+ 1	40			*	70	+ 6	75	− 3	56
?	*			+ 2	76	+ 4	*	− 0	73
*	34			+ 1	70	−48	80	1	75
− 5	30			+ 2	83	− 3	92	+ 5	69
− 8	24			+13	63	+ 3		+12	74
0	39			+ 1	64			+ 4	72
− 3	50			− 9	70			−18	64
+ 5	57			+18	61			+ 1	67
+12	40			− 1				+ 3	71
+ 2	49							− 4	85
− 5								+ 1	

* Break in curve.

From this table we see that the knee-jerk may be large when the tension of the muscles is high, medium, or low, and that the knee-jerk may be small when the tension of the muscle is high, medium, or low. In fact one can trace no constant relation between the amount of tension of the muscles and the size of the knee-jerk. It is interesting to

see how markedly the tension of the muscles may vary without any corresponding alteration in the size of the knee-jerk. This is well seen in Charts II., III. and IV.

In the cases just studied, the tension of the muscles was determined by the position of the line of rest just after the oscillations caused by the preceding knee-jerk had ceased. Ordinarily no further change in the line of rest was seen to occur in the interval elapsing before the next blow was struck. This was found to be the case by experiments in which the records were taken on a revolving drum. Occasionally, however, such changes were seen, and in the following table two cases are reported in which considerable variation in the position of the line of rest occurred in the intervals between the succeeding knee-jerks. Since in these cases the tension of the muscles was changing almost at the moment that the blow was struck, one might expect to find here, if anywhere, that the extent of the knee-jerk was influenced by the tension of the muscles.

Description of Table IV.

The table is headed by the letters representing the names of the two men on whom the examinations were made, and by the numbers of the tables in which these results again appear in this paper. The results of the examinations are given in four columns placed beneath these letters. In the first column is recorded the position of the knee-joint just before the blow was struck; in the second, the extent of the knee-jerk; in the third, the position of the knee immediately after the oscillations had ceased; and in the last column, the change in the position of the knee, occurring in the interval before the next blow was struck.

TABLE IV.

Q [Table VIII. p. 141.]				A [Table IX. p. 142.]			
Position of line of rest just before blow was struck as compared with the original line of rest	Extent of the Knee-jerk	Change of the line of rest from position held just before blow was struck	Further change of line of rest in the interval of quiet	Position of line of rest just before blow was struck as compared with the original line of rest	Extent of the Knee-jerk	Change of the line of rest from position held just before blow was struck	Further change of line of rest in the interval of quiet
0	83	+10	−9	0	53	− 6	+ 1
+ 1	65	+ 4	−3	− 5	40	− 4	+ 1
+ 2	56	+ 2	−1	− 8	41	+ 3	+ 1
+ 3	55	+ 1	−2	− 4	20	− 2	+ 2
+ 2	42	+ 3	−2	− 4	20	− 5	+ 2
+ 3	59	+ 3	−2	− 7	50	+ 4	+ 1
+ 4	26	0	−1	− 2	60	− 3	+ 1
+ 3	53	+ 2	−4	− 4	30	− 2	+ 1
+ 1	36	+ 4	−2	− 5	17	0	+ 1
+ 3	39	0	−2	− 4	39	0	0
+ 1	52	+ 3	−2	− 4	49	+ 4	0
+ 2	42	− 5	−8	0	36	− 1	0
−11	70	+20	−4	− 1	44	+ 8	+ 1
+ 5	32	− 1	−4	+ 8	20	− 1	0
0	54	+ 3	−1	+ 7	55	+ 3	− 1
+ 2	58	− 1	−2	+ 9	33	− 2	0
− 1	50	0	0	+ 7	26	− 3	+ 2
− 1	44	+ 3	−1	− 6	44	+11	− 2
+ 1	32	+ 4	−1	+15	32	+ 8	− 5
+ 4	56	+ 6	−4	+18	26	− 3	− 1
+ 6	47	0		+14	36	− 3	− 1
				+10	36	+ 1	− 1
				+10	26	− 2	− 1
				+ 7	26	− 2	0
				+ 5	33	0	0
				+ 5	24	− 1	0
				+ 4	31	+ 5	0
				+ 9	54	− 5	
				+ 4			

Even in these cases one fails to find any correspondence between the size of the knee-jerk and the changing tension of the muscles, as recorded in the third and fourth columns, or the tension of the muscles compared with the amount which they had at the beginning of the examination, as stated in the first column.

Since the tonus of the muscles is without influence upon the size of the knee-jerk, the tonus theory can hardly be taken as a satisfactory explanation of the dependence of the phenomenon upon the spinal cord. A study of these experiments reveals, moreover, that not infrequently the *flexors* as well as the *extensors* contracted in response to the blow on the ligamentum patellae. This result is a very strong argument in favor of the view that the whole process is reflex. The contraction of the flexors was shown not only by the marked changes in the position of the line of rest which occasionally occurred, but it was plainly visible in the effect which it exerted upon the extent of the oscillations of the leg following the knee-jerk.

If one refers to Table I. and Chart I. he sees that the averages of the oscillations occurring in these extremely regular observations were :—

1st extension = 100, 1st flexion = 50, 2nd extension = 23, 2nd flexion = 8.

In other words, he notices that the first flexion equalled about half the movement of the knee-jerk, the second extension was a little less than half the first flexion, and that the second flexion was a little more than a third of the second extension. A glance at Table V., however, shows one that this proportion is far from constant, even in the case of men who are calm and quiet at the time of the experiment. Table V. has the same form as Table I.

The averages obtained from this table are as follows, viz.:—

	1st Exten.	1st Flex.	2nd Exten.	2nd Flex.
G.	+ 31	− 14	+ 7	− 3
X.	+ 34	− 14	+ 8	− 4
C.	+ 94	− 47	+ 25	− 8
D.	+ 33	− 14	+ 8	− 3

These averages show a general tendency for the second extension to be larger in proportion to the first flexion, than the first flexion to the first extension or the second flexion to the second extension, a result which one would scarcely expect in case the oscillations were caused by the force of gravity and the elastic recoil of the muscles. The size of the second extension suggests that the extensors in these cases did not immediately relax.

The above experiments were upon men who were calm at the time of the examination. This was seldom the case at the first examination. The fact that a stranger was about to experiment

TABLE V.

G, curve 10					X, curve 55					C, curve 23					D, curve 39				
1st Exten. i.e. Knee-jerk +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest	1st Exten. i.e. Knee-jerk +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest	1st Exten. i.e. Knee-jerk +	1st Flex. −	2nd Exten. ÷	2nd Flex. −	Change of line of rest	1st Exten. i.e. Knee-jerk +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest
68	31	14	6	0	29	11	7	3	0	108	42	31	1	+2	45	18	11	3	+2
21	11	6	3	0	29	?	?	?	0	117	59	33	9	0	82	22	15	3	+2
43	19	10	4	0	31	16	9	4	0	146	79	47	17	+1	68	20	13	4	+1
80	37	18	7	0	31	15	9	4	0	122	58	32	8	+2	51	21	11	5	0
65	29	13	5	0	43	19	11	4	0	125	47	26	5	0	27	12	6	3	+1
54	21	10	4	0	31	12	8	3	0	122	61	32	10	0	31	16	8	4	0
41	16	9	4	0	42	18	10	4	0	115	60	33	9	0	21	7	4	2	0
18	8	4	3	0	38	16	9	4	0	128	59	32	9	0	21	9	5	2	0
24	10	6	3	0	46	21	11	5	0	113	61	32	10	−1	32	17	9	4	0
23	10	6	3	0	26	11	7	3	0	110	57	31	?	+1	42	24	13	6	0
30	11	7	3	0	40	17	10	5	0	95	48	25	?	0	57	31	18	8	0
15	7	4	2	0	38	17	10	4	0	79	37	13	4	+1	39	19	10	4	0
19	9	5	3	0	31	12	7	?	0	82	41	19	6	0	21	9	5	2	0
15	7	4	3	0	31	12	8	4	0	93	49	25	9	−1	10	4	2	1	0
16	8	4	3	0	27	11	7	3	0	92	48	26	8	0	21	13	7	3	0
29	14	7	4	0	34	15	8	4	0	76	36	18	5	+1	19	8	5	2	0
20	9	5	3	0	33	15	9	4	0	84	43	22	?	0	24	11	6	3	0
15	6	4	2	0	39	17	10	4	0	81	41	19	6	0	17	8	4	2	0
17	10	5	3	0	34	15	9	?	0	78	39	18	?	0	20	9	5	2	0
24	12	7	4	0	27	11	7	4	0	89	48	24	?	0	37	19	10	5	0
11	5	4	2	0	45	22	12	6	0	45	15	8	?	+1	43	23	13	6	0
					33	15	8	?	0	68	34	16	?	0	31	15	8	3	0
					35	15	8	?	0	50	21	9	?	0	14	6	3	2	0
					35	15	8	4	0						24	11	7	3	0
															15	7	3	2	0
31	14	7	3		34	14	8	4		94	47	25	8		33	14	8	3	

upon them in some unknown way excited nearly all of the men, more or less. When any of them was examined a number of times, or often towards the end of the first examination when the excitement had worn off, the records were similar to those recorded in Table V. This excitement, even though slight, generally sufficed to lessen the oscillations of flexion and to exaggerate those of extension more than in the cases reported in Table V. Examples of this condition are given in Table VI.

TABLE VI.

L, Curve 15.						U, Curve 52.					
No. of Observation	Knee-jerk or 1st Exten. +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest	No. of Observation	Knee-jerk or 1st Exten. +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest
1	102	29	6	5	+ 2	1	81	5	− 2	3	− 2
2	59	31	6	2	+ 1	2	60	32	7	8	− 3
3	51	28	2	?	− 6	3	70	16	11	11	+ 1
4	68	37	7	12	− 5	4	77	11	22	1	+ 7
5	58	29	12	4	+ 1	5	85	+ 8	9	+ 1	+ 2
6	73	18	17	+ 2	+ 6	6	Foot moved spontaneously				− 7
7	81	0	46	+ 14	+ 4	7	55	26	12	8	− 4
8	72	19	18	4	− 1	8	65	?	?	?	− 7
9	72	18	15	4	+ 1	9	95	6	12	+ 5	+ 6
10	72	+ 2	39	?	+ 6	10	94	4	37	6	+ 4
11	64	14	21	4	− 1	11	75	9	30	3	+ 2
12	75	8	12	1	+ 1	12	96	14	4	3	− 1
13	72	8	14	3	− 1	13	79	?	?	?	+ 8
14	66	18	18	?	?	14	88	+ 5	56	+ 7	+ 15
15	Break in curve					15	81	21	38	+ 4	+ 6
16	70	12	16	+ 1	+ 2	16	75	13	39	0	+ 4
17	76	6	14	0	+ 1	17	Break in curve				− 48
18	70	10	27	+ 1	+ 2	18	80	0	20	6	− 3
19	83	7	27	+ 11	+ 13	19	92	11	35	+ 1	+ 3
20	63	1	3	?	+ 1						
21	64	26	0	?	− 9						
22	70	+ 3	36	+ 14	+ 18						
23	61	7	9	2	− 1						

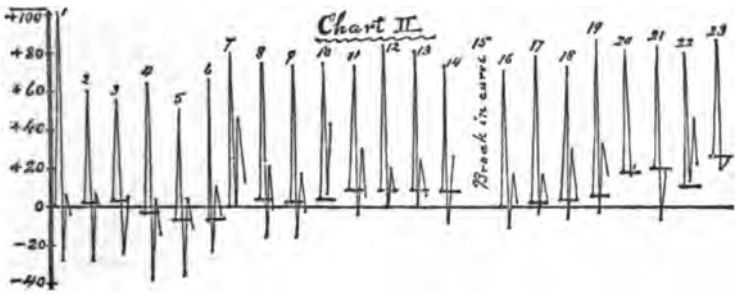
Thus in the case of L, curve 15, every observation, except number 21, from number 6 through the table, shows that the first flexion was small in proportion to the first extension, the second extension was not only large in proportion but frequently larger than the first flexion, and the second flexion was very small. In observations 10 and 22 the

first flexion did not even get back to the line of rest, and the same is true of the second flexion in observations 6, 7, 16, 17, 18, 19 and 22.

Examination of U, curve 52, shows a similar state of things. There are the same excessive oscillations of extension and small oscillations of flexion, in a large proportion of the observations, while the first oscillation of flexion does not get back to the line of rest in observations 5 and 14, and the second flexion does not get back in numbers 5, 9, 14, 15 and 19.

These peculiarities are well seen in the case of L, curve 15, recorded in Chart II.

L, Curve 15.



One cannot observe the tendency shown in these cases for the extensor muscles to check the oscillations of flexion and to increase the oscillations of extension, without noticing the remarkable exceptions to this tendency. Thus in L, curve 15, one sees the second extension to be very small in observations 1, 2, 3 and 4, and in observation 1 the second flexion is nearly as large as the preceding extension, while in observation 4 it is even larger. In U, curve 52, there is a still more interesting exception, for in observation 1 the second extension does not get back to the line of rest and the second flexion is larger than the second extension.

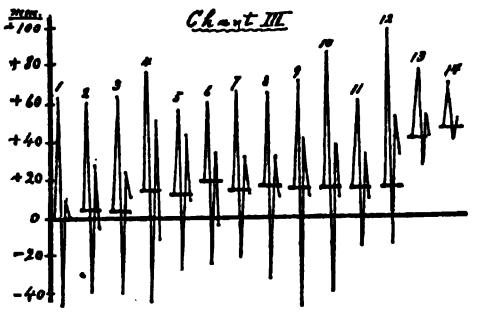
These exceptions are of the greatest interest, because they can scarcely be explained except by assuming a contraction of the flexor muscles of the knee. Such observations are of frequent occurrence and are unquestionably accurate. In certain cases, indeed, the contraction of the flexors is so strong as not only to check the second extension but to lessen the extent of the knee-jerk. C, curve 16, recorded in Table VII. and Chart III. is a case in point. The first flexion is disproportionately large in most of these experiments, and in observations 6, 8, and 9 it is as large, or even larger than the knee-jerk.

TABLE VII.

C, Curve 16.

No. of Observation	Knee-jerk or 1st Exten. +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest
1	63	46	9	0	+ 5
2	56	45	23	10	− 1
3	60	44	20	+ 7	+ 11
4	61	58	36	27	+ 2
5	44	39	31	4	+ 7
6	41	44	14	24	− 5
7	52	35	16	2	+ 2
8	49	49	18	5	− 1
9	55	62	24	5	0
10	70	56	22	5	0
11	45	30	17	6	0
12	82	31	36	+ 15	+ 25
13	36	13	11	+ 2	+ 5
14	24	6	5	?	+ 2

C, Curve 16.



The size of the second extension and the second flexion together with the shape of the curve, shows that the extensors as well as the flexors were actively contracting. In cases such as the above one sees the knee-jerk, though beginning rapidly and vigorously, to be suddenly checked and changed to flexion, which movement, as has just been shown, may be even greater than the knee-jerk. This result corresponds with the observations of certain physicians, who have told me that they have come to doubt the extent of the knee-jerk as an accurate measure

of the activity of the process, and that they were in the habit of basing their conclusions upon the rapidity and "jerkiness" of the phenomenon as well as upon the amount of the movement.

The fact that both flexors and extensors may be excited by the blow on the ligamentum patellae, or its results, is shown in still other cases where the oscillations of extension and flexion instead of being long and pendulum-like are short and jerky in character, so that they remind one of clonus. In these cases it is probable that both of the antagonists are actively contracting; and the leg oscillates as if swinging between two tightly stretched elastic bands. This is illustrated by the experiments recorded in Table VIII.

TABLE VIII.

Q, Curve 29[a].

No. of Observation	Knee-jerk or 1st Exten. +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest	Further change of line of rest
1	83	15	14	+ 7	+ 10	− 9
2	65	12	9	+ 3	+ 4	− 3
3	56	7	7	0	+ 2	− 1
4	55	12	4	1	+ 1	− 2
5	42	3	8	+ 2	+ 3	− 2
6	59	2	11	+ 4	+ 3	− 2
7	26	9	4	3	0	− 1
8	53	3	6	+ 1	+ 2	− 4
9	36	3	10	1	+ 4	− 2
10	39	5	4	1	0	− 2
11	52	4	8	1	+ 3	− 2
12	42	9	1	7	− 5	− 8
13	70	+ 29	26	+ 20	+ 20	− 4
14	32	6	7	2	− 1	− 4
15	54	5	7	+ 2	+ 3	− 1
16	58	10	3	3	− 1	− 1
17	50	2	8	0	0	0
18	44	2	7	+ 3	+ 3	− 1
19	32	3	9	+ 3	+ 4	− 1
20	56	8	3	+ 6	+ 6	− 4
21	47	6	9	0	0	

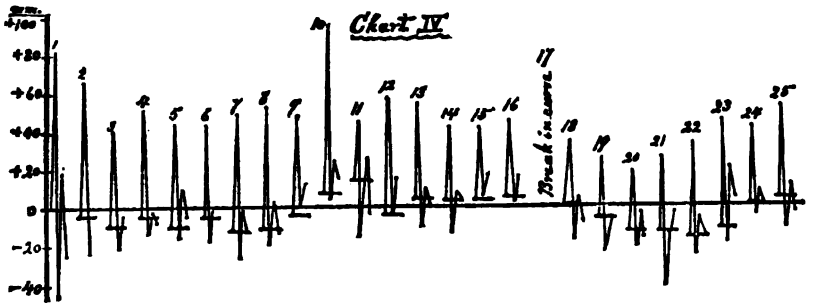
The curves recorded in this table show many irregularities, and there were other examinations made in which no prevailing tendency could be observed, but every possible variation was found. These peculiar results are explained by the fact that sometimes the flexors and sometimes the extensors of the knee exert the controlling influence. A curve 14, recorded in Table IX. and Chart IV. is a good example of the eccentricities possible to the knee-jerk.

TABLE IX.

A, Curve 14.

No. of Observation	Knee-jerk or 1st Exten. +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest
1	81	45	18	24	− 4
2	70	29	?	?	− 5
3	50	13	4	?	+ 5
4	56	17	2	4	− 5
5	53	6	18	+ 5	+ 5
6	48	14	2	?	− 7
7	61	14	10	+ 1	+ 1
8	63	8	13	3	+ 8
9	51	+ 4	15	?	+ 11
10	88	5	17	+ 7	+ 7
11	30	30	11	19	− 18
12	60	0	18	?	+ 7
13	50	14	5	3	− 1
14	39	18	4	0	0
15	37	+ 1	12	?	+ 1
16	40	2	12	?	?
17	Break in curve				
18	34	17	4	7	− 5
19	30	18	0	?	− 8
20	24	8	10	3	0
21	39	29	0	10	− 3
22	50	8	10	1	+ 5
23	57	9	31	+ 15	+ 12
24	40	7	6	0	+ 2
25	49	14	7	7	− 5

A, Curve 14.



The first oscillation of flexion was prevented from returning to the line of rest in observations 9, 12 and 15. The same is true of the oscillation of second extension in 19 and 21. The first oscillation of flexion is disproportionately large in 11 and 21. The second extension is larger than the first flexion in a large proportion of the cases, yet the second flexion is larger than the second extension in 1, 4, 11, 18 and 21. The second flexion does not attain the line of rest in 5, 7, 10 and 23.

The results of these experiments can leave no doubt that under certain circumstances, at least, the flexors as well as the extensors are thrown into contraction by the blow on the ligamentum patellae or its sequellae. This discovery is of great importance not only as enabling us to understand the character of the knee-jerk, but also as a hint as to the methods of muscular co-ordination. It has long been thought that antagonistic muscles both act at the same time and that all the movements of the limbs are the result of finely graded differences in these opposing forces. I discovered while working in Leipzig[1], that all the muscles of the frog's leg will respond nearly simultaneously to a strong reflex stimulus, and it seems to me that the experiments reported in this paper favor the view that the same thing is true of co-ordinated muscular actions in man. It is probable, however, that the contraction of the antagonist is well developed only when the action is vigorous or extended.

There can be no doubt that the blow on the ligamentum patellae resulted in a contraction of the flexor as well as of the extensor muscles in many of my experiments. How were the flexors stimulated, reflexly, or directly by the strain brought upon them by the extension of the knee?

[1] "Die räumliche und zeitliche Aufeinanderfolge reflectorisch contrahirter Muskeln." *Archiv fur Anat. u. Physiol.*, Physiol. Abthl., 1885.

I am inclined to think that the extensors and flexors are both irritated by the same process. The twitch of the tendon caused by the blow on the ligament probably irritates the ends of the centripetal nerves lying in the tendon and muscle, and these nerve ends develop a stimulus which excites the reflex centres of the spinal cord. How many reflex centres are affected and how strong a reflex action is developed depends on the strength of the irritant and on the condition of the reflex mechanisms at the time. When the person is calm and quiet, only the extensors appear to be stimulated, but when he is excited, the flexors, as well, receive reflex stimuli. The extensors are irritated first and their contraction is under way before the flexors begin to contract; nevertheless, the difference in time is not great, as is shown by the fact that the contraction of the flexors often follows that of the extensors with sufficient rapidity to prevent the completion of the movement of extension. Thus one may see at the beginning of a series of experiments, when the man is excited, very small knee-jerks though the movements are sudden and violent. Gradually, as the man becomes calmer, the knee-jerks become longer and less jerky, and, finally, as he quiets down still more, they get small again. The effect of the excitement was to cause an active contraction of the flexors almost simultaneously with that of the extensors, and this antagonistic action checked the knee-jerk before completion. As the excitement became less, the flexors were stimulated less, and the contraction of the extensors was able to show itself in a larger knee-jerk. When the subject had become calm, the extensors alone were irritated, and they received a milder stimulus, so that the knee-jerk was small again. Such was the condition in the case of C, curve 3, recorded in the following Table, X.

This explanation is in harmony with what we know of reflex actions, in general. A slight irritation will call out a reflex contraction of a few muscles, and a stronger irritant applied to the same part will excite a stronger contraction of these muscles and at the same time stimulate certain other muscles. The muscles which respond to the least stimulus also contract first when more muscles are excited by the stronger irritant.

It may be asked, however, is it not possible that the contraction of the flexors is due to a direct mechanical stimulation of the muscle fibres by the sudden strain brought upon them by the rapid extension of the knee? There are several reasons why this explanation is unsatisfactory. First of all one remembers that the flexors often contract very actively when they have been subjected to a very slight strain,

TABLE X.

C, Curve 3.

No. of Observation	Knee-jerk or 1st Exten. +	1st Flex. −	2nd Exten. +	2nd Flex. −	Change of line of rest
1	47	3	6	8	− 4
2	Trembling of muscles				+ 3
3	59	37	12	+ 1	+ 7
4	72	65	3	7	− 4
5	69	69	− 10	13	− 2
6	76	27	17	1	+ 5
7	96	56	42	+ 17	+ 14
8	107	52	52	+ 2	+ 2
9	Trembling of muscles				+ 11
10	96	.61	48	+ 13	+ 20
11	67	47	40	+ 7	+ 22
12	24				
13	Break in curve; start again				
14	82	44	11	5	+ 2
15	54	19	5	6	− 3
16	40	60	22	9	+ 2
17	27	54	− 1	24	− 10
18	Foot moves spontaneously				+ 2
19	63	56	31	10	+ 4
20	40	54	29	5	+ 8
21	71	53	33	?	+ 2
22	45	25	13	1	+ 2
23	37	29	11	4	0
24	55	44	17	5	0
25	45	22	9	2	+ ·1
26	54	46	20	8	− 1
27	53	33	16	3	+ 1
28	57	41	19	12	− 3

and that a slight strain is unable to excite a muscular contraction. In the case of the extensors, for instance, we find that depression of the ligamentum patellae even if sudden when performed by the fingers does not cause a knee-jerk; nor does sudden flexion of the knee, when produced by the hand. The still greater suddenness or jar of a blow on the ligament is necessary to the development of the phenomenon. It is probable that the same is true of the flexors, and certainly we find that sudden extension of the knee by the hand does not cause

contraction of the flexors. The strain brought on the flexors by the extension of the knee must be a very gradual one, and not to be compared with the twitch which a blow on the ligament would produce. Moreover, the flexors often contract when the foot has made only a slight movement. Thus in observation 11, of A, curve 14, in Table IX., the foot had moved only 30 millimetres when the extension was checked by the contraction of the flexors. To have checked the knee-jerk at this point the contraction of the flexors must have started when the foot had moved even less than this distance. Such a slight movement of the foot would be accompanied by a stretching of the long flexor muscles of only at the most three or four millimetres. Nor is this an isolated example. In case of C, curve 3, Table X, observation 15 gives the movements—+ 27, − 54, − 1, − 24, that is to say, the extensors had only extended the foot 27 millimetres when a violent contraction of the flexors occurred which stopped the knee-jerk short and produced a backward swing of the foot of 54 millimetres. Many other, though perhaps less striking examples, are recorded in the table.

One can scarcely believe that such a slight strain would cause an active contraction of the flexors when he sees in the same examinations that a much greater strain has no such effect. It may be said that this argues as well against the theory of reflex as of peripheral irritation. This is not so, however, because we have evidence that the reflex centres are continually undergoing changes in irritability, while as far as we know the irritability of the muscles does not change much within short intervals of time.

I have already called attention to the fact that the flexors sometimes not only check the contraction of the extensors, but actually contracted further than the extensors had done. It is hard to understand how a muscle by contracting can irritate its antagonist to a stronger contraction than it itself made, unless indeed its antagonist was the more irritable of the two. This explanation will not suffice, because the excessive contractions of the flexors only appear as exceptions, and as a rule the contractions of the extensors are much the larger.

Summary of Results.

There are two theories of the nature of the knee-jerk; the one regards it a reflex action, the other a purely peripheral process. The reflex theory readily explains the intimate dependence of the phe-

nomenon upon the spinal cord, and the time argument, owing to our meagre knowledge of reflex times, in general, is inconclusive. The peripheral theory is not tenable, because the explanation which it offers of the dependence of the knee-jerk upon the spinal cord is unsatisfactory. It assumes that muscle-tonus is continuous, and that the irritability of the muscle to mechanical stimuli is dependent on its tension; both assumptions are without proof. Moreover, the theory is opposed by many facts.

The knee-jerk may be present when muscle tonus appears to be wanting, and may be absent in the case of men who apparently have a normal amount of tonus. When the knee-jerk is lacking it cannot be restored by any amount of tension which can be artificially supplied to the muscle.

The tonus theory does not explain the difference which always exists in the size of the successive knee-jerks, for it is found experimentally that the size of the knee-jerk is not influenced by slight variations in the tension of the muscle; nor can the changes in the amount of the knee-jerk be attributed to alterations of the irritability of the muscle dependent on fine variations in tonus, because experiments show that the irritability of the muscle does not change within short intervals of time.

The peripheral theory does not explain the reenforcements of the knee-jerk, because reenforcing acts, unless very violent, do not alter the tension or irritability of the muscles.

The discovery of Mitchell and Lewis, that muscular contractions called out by electrical stimulation cannot be reenforced, is inexplicable by the peripheral theory though readily explained by the reflex theory.

Finally, occasionally the flexors, as well as the extensors, of the knee are seen to contract in response to the blow on the ligamentum patellae. This contraction of the flexor muscles is of reflex origin, and there is little reason to doubt that the extensors are irritated by the same reflex process. The idea that the flexors are mechanically stimulated by the strain brought on them by the sudden extension of the knee is untenable, because we know that muscles are not irritated by slight strains on their tendons, and the flexors are seen to contract when the knee has extended so little as to bring almost no strain upon them; moreover, in spite of the fact that muscle irritability does not change within short intervals of time, small knee-jerks may be seen to be accompanied by marked contractions of the flexors, and, immediately after, large knee-jerks by little or no flexor contraction.

Since only the reflex theory can satisfactorily explain the influence of the central nervous system over the extent of the knee-jerk, we must adopt it, and must look to future experimental work on reflex times to remove the apparent contradiction involved in the rapidity of the process.

ON THE NORMAL DURATION AND SIGNIFICANCE OF THE "LATENT PERIOD OF EXCITATION" IN MUSCLE-CONTRACTION. [AN ADDENDUM AND CORRECTION.] BY GERALD F. YEO.

PROFESSOR von Regéczy, of Budapest, informs me in a private letter, that some erroneous conceptions of his work[1] appear in my paper[2] in the last volume of this Journal.

I have much pleasure in taking the earliest opportunity of correcting the mistakes and of apologising to him for having misunderstood his methods. I have also to thank him for the courteous manner in which he has called my attention to the following points.

In describing his method of testing the elongation of the spiral spring he says, (page 590), "jetzt ziehe ich das obere Ende der Spirale "heftig an, welches Moment, sowie die Dauer der Handbewegung ein "Depréz'sches Chronograph, dessen Strom durch eine Stimmgabel von "100 Schwingungen unterbrochen wird, gleichfalls auf den Cylinder "aufzeichnet."

In the absence of further detail I imagined the movement was made by the hand, and I wrote (page 434—435) that he "tests the elongation "of a spiral spring by the graphic method. With a writing point "attached to the weight and a Depréz signal to record the hand-motion, "he makes a tracing on a rapidly rotating drum while he jerks the "upper end of the spring." And on the same page I remark that my method, i.e. using a frog's muscle to move the spring "has at least the "advantage of being free from adventitious hand-jerks."

He now sends me a diagram of his method and explains that "das "Anziehen der Spiralfeder geschah durch das schnelle Herunterdrücken "eines als stromschliessenden Schlüssel dienenden Hebels, welcher in "der anfänglichen Stellung den electrischen Strom vom Stimmgabel "und vom Chronographen abblendete; diese Abblendung wurde wäh-

[1] *Pflüger's Arch.*, Bd. XLIII., S. 584. [2] Volume IX, p. 306.

"rend der Hebelbewegung aufgehoben, somit war bei meiner Methode "von 'adventitious hand-jerks' keine Rede."

Concerning the lithograph of the myogram appended to his work I say (p. 436), "From a careful examination of the curves given by "Regéczy it does not appear at all clear to me that the depressions "depend on stimulation of the muscle.

"In all but one of these myograms in which the elongation occurs, it "begins at varying distances before what is marked as the point of "stimulation, and the depression is visible in eight out of ten tracings "though from the context only five were stimulated below, and therefore "only that number should have shown the elongation.

"I have often observed similar irregular depressions in the muscle "curve preceding or during the latent period, and have thought them to "be caused by accidental oscillation of the lever."

Prof. von Regéczy has now kindly sent me some of his original photograms, including that from which the plate in question was taken.

Had I seen these instead of the lithograph I certainly should not have made the above remarks, which though true of the lithograph are not so of the originals. In these the interlatent depression in each curve is most definite and regular; it commences about the middle of the latency and is quite absent in those curves in which the stimulation was applied at the upper part of the muscle.

I am glad Prof. von Regéczy sees that the lithograph is to blame for he says in his letter, "Was jene Curven anbelangt welche in "Lithographie wiedergegeben sind, so machen dieselben—wie im allge-"meinen die lithographische Leistungen—auf Pünktlichkeit keinen "Anspruch."

I am extremely sorry that this unlooked-for inaccuracy in the lithograph prevented my corroborating the opinion of the Author, and caused me to appear to question the logic of his conclusions, which I now learn from his beautiful original curves are based on very clear and convincing evidence.

He was also good enough to call my attention to the fact that on page 430 of my paper the number ·0098″ is printed in error, as the mean latency for the whole muscle, instead of ·0089″ the mean of the figures, ·0082″ and ·0096″, given by him (page 606).

He considers the extreme variations, which I mention in the same paragraph as being excessive, do not at all impugn the reliability of his

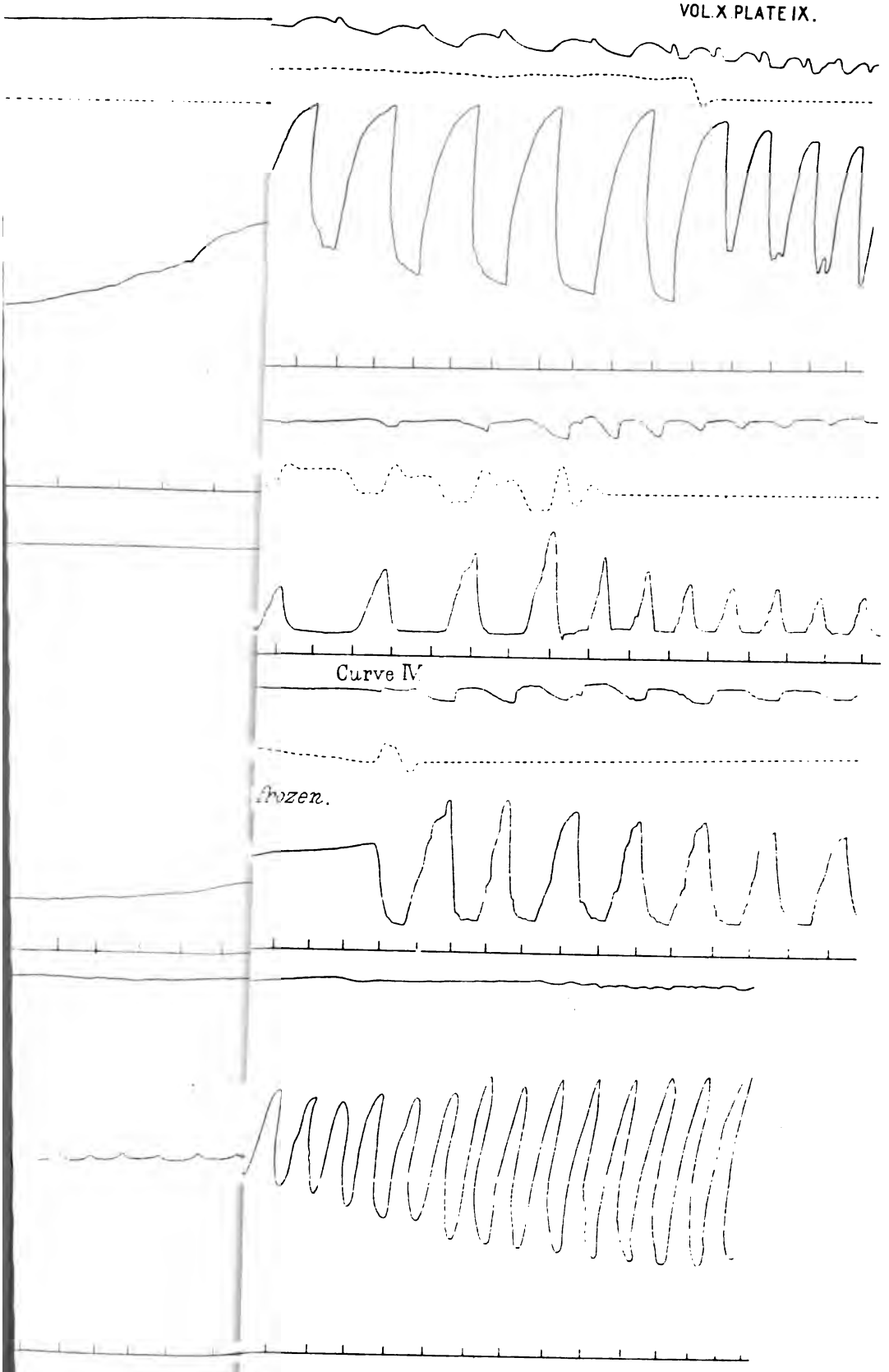
method, because the stimulation was applied in different ways to different parts of the muscle.

Instead of saying that "in one series of experiments" the latency varied between ·0052″ and ·0033″, I should have given separately the variations of the two parts of his series (page 604) thus:

When stimulated above the latency varied between ·0152″ and ·0075″; when stimulated below it varied between ·0129″ and ·0036″.

The variations from ·0045″ to ·0019″ named in the same paragraph of my paper occurred in a series of experiments in which the muscle was alternately stimulated, at both ends synchronously, at one end only, or in the middle. The difference of method Prof. von Regéczy considers quite explains this variation of the latency.

In determining the latency of the transverse thickening of the muscle I found that stimulation in the middle, at the extremities, or at both parts simultaneously did not cause any such variations.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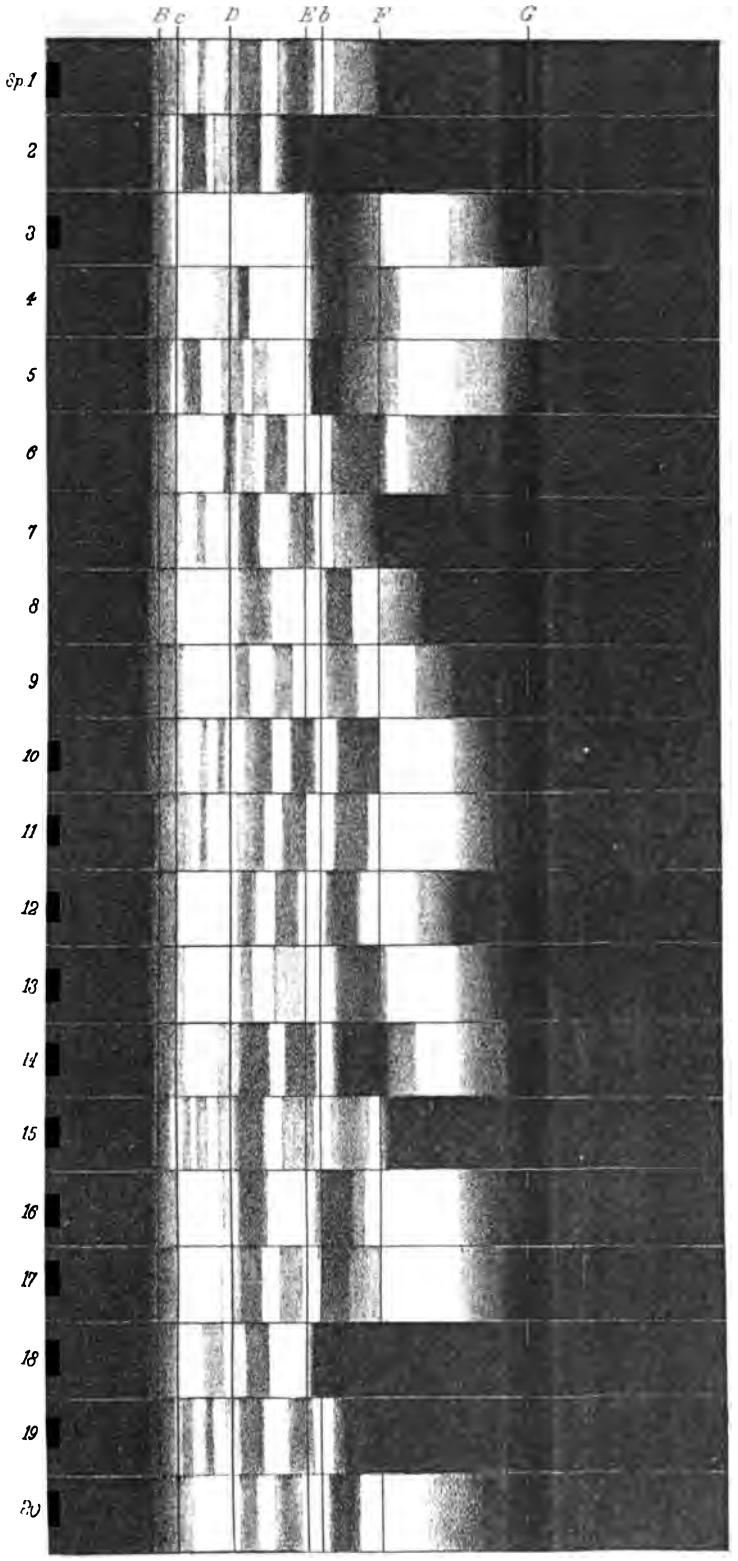

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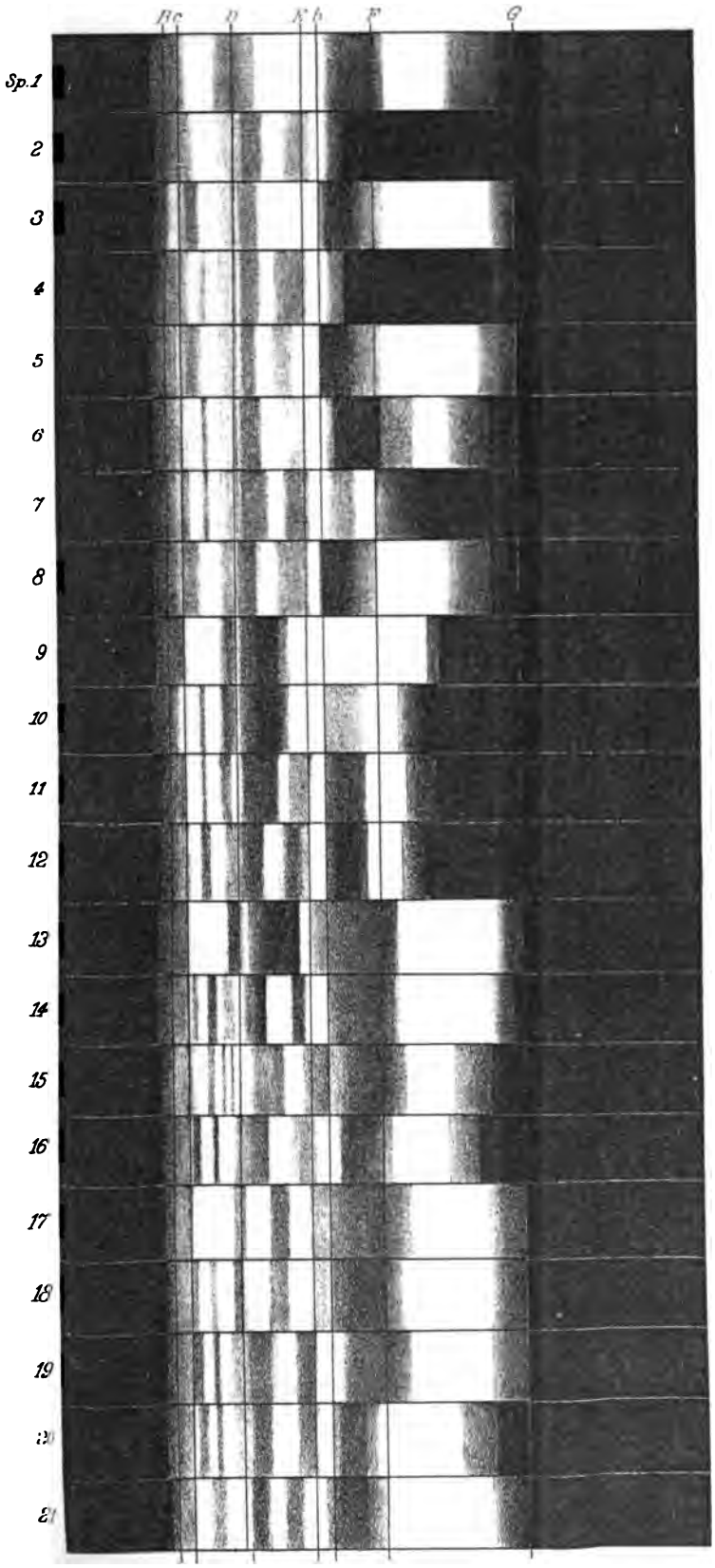

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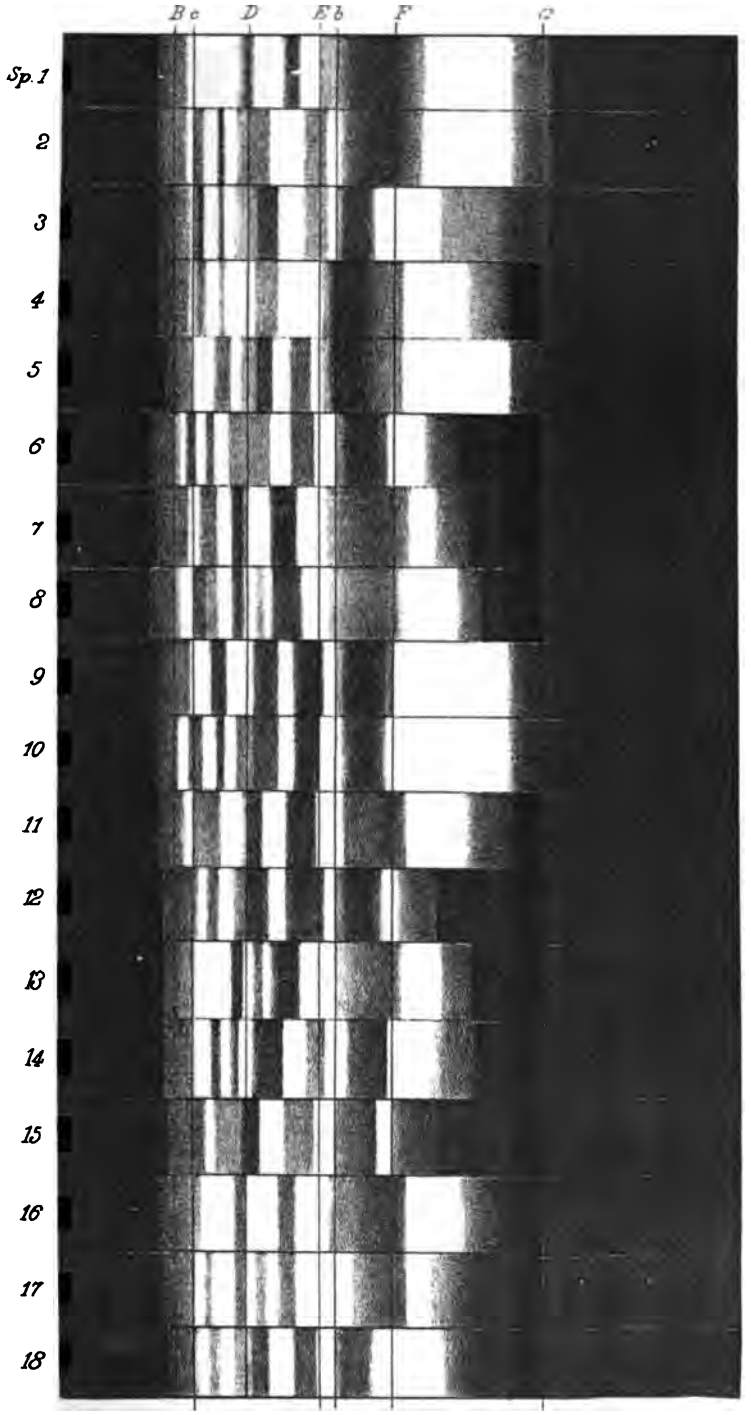

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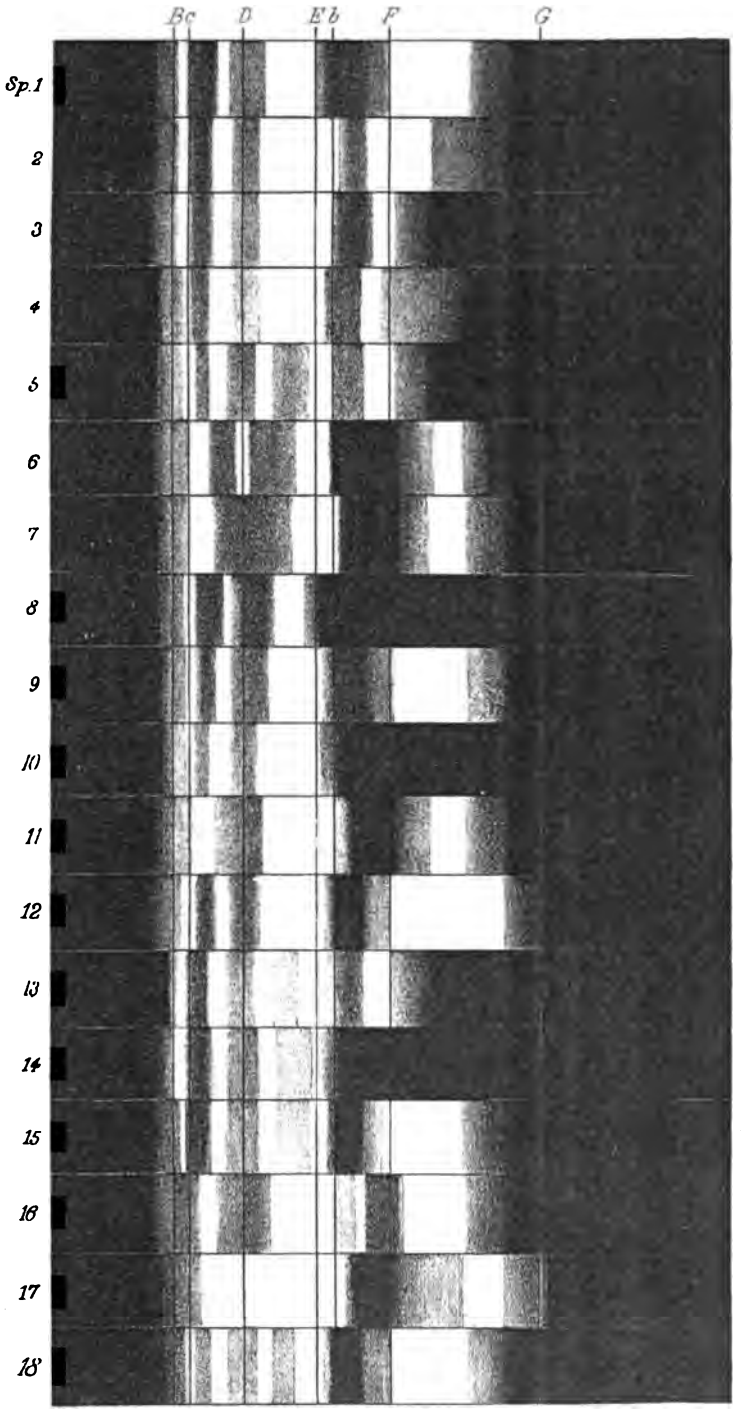

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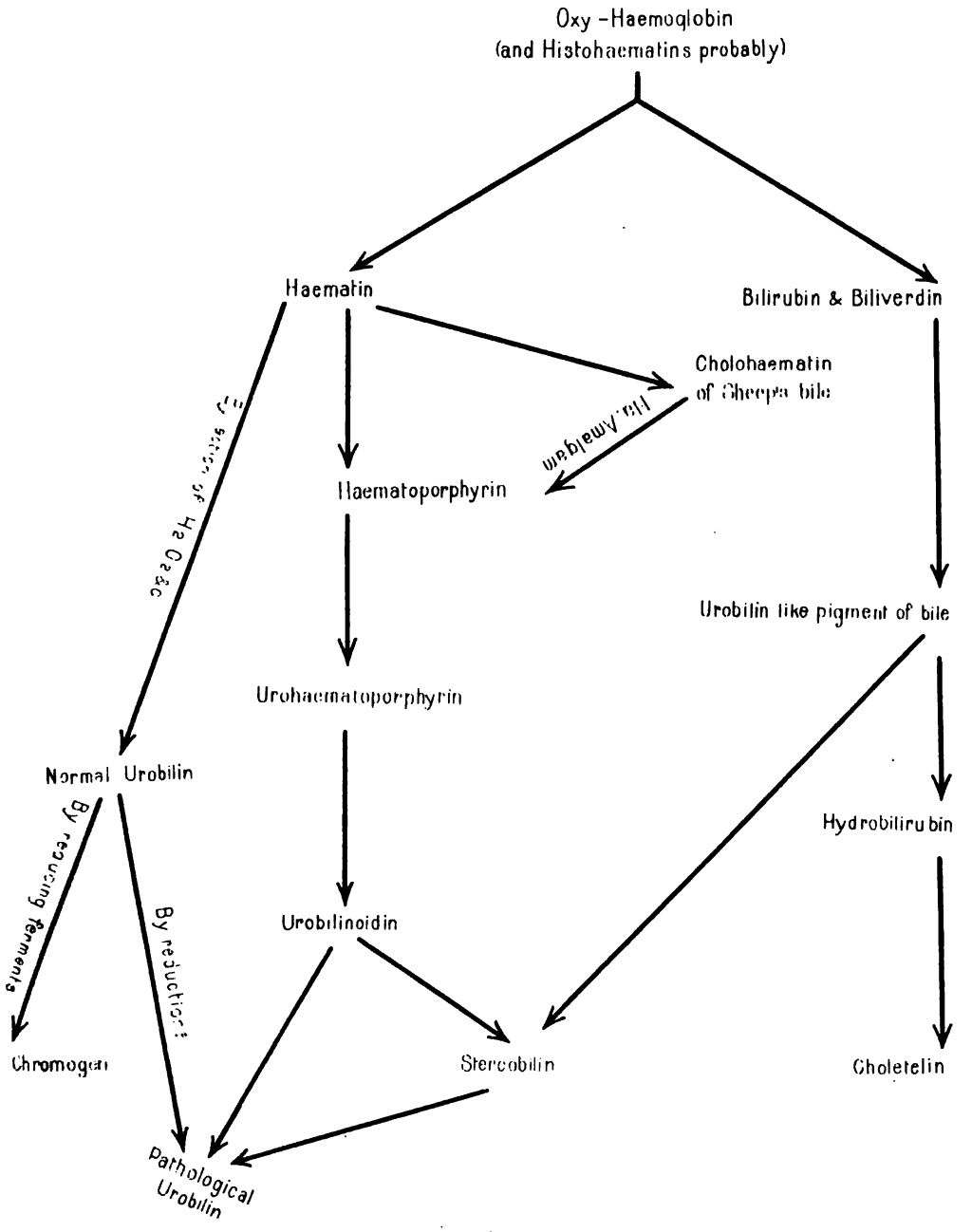
CHART V



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ON THE RELATION BETWEEN THE STRUCTURE, FUNCTION, DISTRIBUTION AND ORIGIN OF THE CRANIAL NERVES; TOGETHER WITH A THEORY OF THE ORIGIN OF THE NERVOUS SYSTEM OF VERTEBRATA. By W. H. GASKELL, M.D., F.R.S.

(Plates XVI.—XX.)

A preliminary account of the first part of this paper is published in the Proceedings of the Royal Society, Vol. 43, *p.* 382, *and in the Medico-chirurgical Transactions, Vol.* 71, *p.* 363; *and a condensed account of the second part in a paper read before the Anatomical Society on June* 23, 1888, *and published in the Journal of Anatomy and Physiology, Vol.* 23.

PART I.

On the relation between the structure, function, distribution and origin of the cranial nerves.

In a former paper[1] I pointed out that the conception of a spinal nerve as described there, removed many of the difficulties which have hitherto hindered the comparison of the cranial with the spinal nerves; in this paper I propose to compare structure and centres of origin as regards the cranial with the spinal nerves, more closely than I was able to do at that time. In order to make such a comparison with any chance of success, it is necessary in the first place to have a clear conception of the elements composing a typical spinal nerve; in addition it is most essential to understand as far as possible the centres of origin of these different elements, for I am convinced that the true morphological meaning of the segmental arrangement of nerves whether spinal or cranial is bound up with the arrangement of the groups of nerve cells in the central nervous system, rather than with the exits of the different nerves from the central nervous system. It is therefore advisable to

[1] This *Journal*, Vol. VII. p. 1.

commence the study of the cranial nerves with a sketch of the way in which I consider a typical spinal nerve to be built up.

The conclusions to which I came in my former paper were, that in addition to the well-known division into afferent and efferent groups of fibres, two other divisions existed in the nervous system; the one, a division of nerve fibres and their centres of origin into somatic and splanchnic groups; and the other, a division into anabolic and katabolic groups. The most essential division of the nervous system is that expressed by the terms splanchnic and somatic, for it embraces the minor divisions of afferent and efferent and of anabolic and katabolic. The characteristics of this division are to be found equally well in the cranial as in the spinal nerves. The division is a natural one which includes not only the nervous system but the whole organism, for by somatic nerves are meant those nerves which supply structures derived from the epiblast and from that part of the mesoblast which forms the mesoblastic somites, and by splanchnic nerves those which supply structures derived from the hypoblast and the rest of the mesoblast; in accordance with this phraseology we find in the body two separate segmentations, the one "somatic" (called by Ahlborn Mesomeric), which corresponds to the "dorsal segmentation" of v. Wijhe and Gegenbauer, and is characterised by the formation of somites, and the other "splanchnic" (called by Ahlborn Branchiomerie), the "ventral segmentation" of v. Wijhe and Gegenbauer, more local in situation characterized by the formation of gill clefts.

In my former paper I have pointed out how the splanchnic nerves are divisible from the somatic in so far as the structure and origin of their efferent fibres are concerned; the separation of the afferent somatic and splanchnic nerves is a more difficult matter and one which will require a large amount of time for its successful accomplishment. Leaving the afferent fibres for future consideration, we see that the fibres of an efferent root are divisible into a ganglionated and a non-ganglionated group of which the former belongs to the splanchnic system, while the latter is partly splanchnic and partly somatic.

The somatic efferent nerves, which contain the large medullated motor fibres of those striated muscles which arise from the muscle plates, spring from groups of nerve cells (A in Fig. 1) situated in the anterior horn; the characteristic appearance of these nerve cells is shown in the Figure on p. 352 of Schwalbe's *Neurologie*, where also it is asserted that they vary in size from 67—135 μ.

The splanchnic efferent nerves on the other hand are in all proba-

bility connected with the cells of Clarke's column (C in Fig. 1), the cells of the lateral horn (B and E in Fig. 1), and with the solitary cells of the base of the posterior horn (D in Fig. 1).

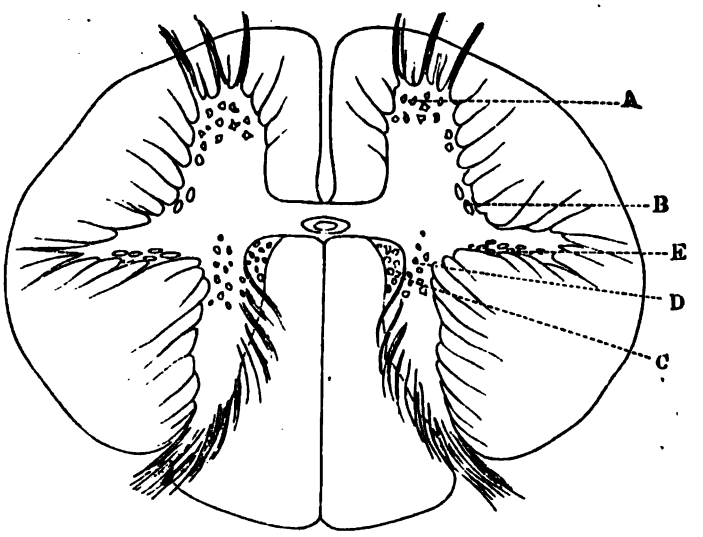

FIG. 1. Section of the spinal cord in the thoracic region.
(*Slightly modified from Schwalbe.*)

The characteristic appearance of the cells of Clarke's column is well shown in the Figure on p. 58 of Henle's *Nervenlehre des Menschen* (2nd Edition). They measure from 45—90 μ, according to Stilling and Schwalbe.

The cells of the lateral horn in the thoracic region, i.e. the cells of the intermedio-lateral tract (E in Fig. 1), are described by Schwalbe (p. 349) as consisting of groups of spindle-shaped multipolar cells which are smaller than those of the anterior horn and are arranged for the most part with their long axis in the direction of the lateral horn.

The solitary cells of the base of the posterior horn are figured in Schwalbe (p. 350) as elongated spindle-shaped multipolar cells of a size much smaller than those of the anterior horn, possessing a marked axis cylinder process the direction of which is nearly at right angles to the long axis of the cell.

We see then that these different groups of nerve cells present marked characteristics and (see Fig. 1) are arranged in a definite manner in the spinal cord; those groups of nerve cells which in this

paper are supposed to give origin to the splanchnic efferent nerves being collected together around the central canal, while the centres for the somatic efferent nerves are situated in the outlying anterior horns of grey matter.

Further, in order to understand the origin of the cranial nerves it is advisable to consider somewhat more closely the meaning of the different groups of nerve cells situated in what I may now call the splanchnic region of the grey matter, and as unfortunately these different groups of cells have not been traced directly into nerve fibres of definite function the only evidence for their function must of necessity be indirect and consequently somewhat speculative. I do not therefore look upon the following remarks as anything more than suggestions which may help to the elucidation of the difficult problem before us.

We have to consider the nerve cells of three different groups, viz.:

1. Cells of Clarke's column.
2. Cells of lateral horn.
3. Solitary cells of base of posterior horn.

In my previous paper I pointed out (1) that the cells of Clarke's column were associated with the presence in the anterior roots of bundles of very fine medullated fibres forming the splanchnic ganglionated efferent nerves; (2) that the solitary cells of the base of the posterior horn increased in number to form part of the vagus nucleus, and (3) that the cells of the lateral horn belonged to the same group as those of the nucleus of the facial &c., and formed the motor nerve cells of those splanchnic non-ganglionated nerves which supplied the voluntary muscles derived from the lateral plates of mesoblast,—muscles which correspond closely with A. Schneider's group of "visceral muscles."

Cells of Clarke's column.

We can I think go further than this by means of the process of elimination. Thus, the cells of Clarke's column are found in the cranial, thoracic, and sacral splanchnic regions; consequently they do not give origin to the vasoconstrictor nerves,—i.e. the katabolic nerves of the muscles of the blood vessels,—which arise only in the thoracic region, and with the vasoconstrictor nerves the katabolic glandular nerves are in all cases closely associated, so that these two categories of nerves may be eliminated from the consideration of Clarke's column. Further, Clarke's column is most developed from the 9th dorsal to the 3rd lumbar nerves, therefore it is associated with the abdominal

splanchnics rather than with the cervical splanchnics. Moreover, if we remove from the abdominal splanchnics the katabolic nerves of the glands and blood vessels, we have left all the anabolic nerves and the katabolic nerves of the muscles of the alimentary tract. We know however that the vagus nerve is the great provider of these latter nerves, i.e. the viscero motor nerves, and that it is not easy to prove that the abdominal splanchnics contain any nerves which cause contraction of the muscles of the alimentary canal; we know on the other hand that the great function of these splanchnic nerves is to inhibit the movements of the alimentary tract. The natural conclusion is that the cells of Clarke's column are associated with anabolic rather than katabolic nerves and that they give origin to the inhibitory or anabolic nerves of the muscles of the alimentary canal, and perhaps also to the corresponding nerves of the vascular and glandular systems. This conception is in harmony with their presence in the sacral region in connection with the *n.n. erigentes*, and at the upper part of the cervical region where the inhibitory cardiac nerves are given off. Again, the observations of Mott show that the cells of Clarke's column are not all alike, but are divisible into two groups, of different sized cells, which suggests that the nerve fibres arising from these two groups of nerve cells supply more than one special structure.

Solitary cells of base of posterior horn.

We come next to the solitary cells of the base of the posterior horn which I was inclined in my former paper on the strength of Schwalbe's suggestion to classify as splanchnic sensory nerve cells. The evidence for their connection with sensory nerves as given by Schwalbe (p. 353) is not strong, being based mainly on the observation of Freud that the posterior cells (Hinterzellen) of Petromyzon which he considers to be homologous with these solitary cells, send their axis cylinder processes into the posterior roots. Against this observation is to be reckoned in the first place that no posterior root fibres in the higher vertebrata are known to arise as axis cylinder processes of ganglion cells in the central grey matter; secondly, as Schwalbe points out, the observations of Gerlach show that the axis cylinder processes of these solitary cells pass direct into the anterior horn and so form anterior root fibres and not as Deiters thought fibres of the posterior roots. Finally the observations of Freud are not necessarily contrary to the motor character of these cells even if they are homologous with the Hinterzellen of Petro-

myzon, for the observations of Ransom[1] and D'Arcy Thompson show that the posterior root of the Petromyzon contains the small fibres with their ganglia which correspond to the so-called sympathetic system in other vertebrata, in other words contains ganglionated splanchnic nerves closely corresponding to those which are in the higher vertebrata essentially efferent in character. Freud's observation therefore harmonises with the motor character of these ganglion cells on the possible assumption that in Petromyzon the nerve roots are arranged not as afferent and efferent but as ganglionated and non-ganglionated; the ganglionated containing both afferent and efferent ganglia.

Again, if the observations of His[2] are confirmed, it is very significant that the jugular ganglion should in the embryo be connected only with the nerve fibres forming the ascending root of the vagus or *fasciculus solitarius*, while the fibres arising from the cells of the vagus nucleus pass not into this ganglion but into the ganglion trunci vagi and so to the vagus nerve trunk. In accordance with my previous observations this means that the afferent vagus fibres all arise from the ascending root of the vagus, just as all the afferent fibres of the trigeminal arise from the ascending root of the trigeminal; that therefore the cells of the vagus nucleus give origin to efferent and not to afferent nerves.

A similar conclusion has been arrived at by Osborn[3] in a recent paper on the internal structure of the Amphibian brain in which he gives reasons for considering the nucleus of the vagus in the floor of the 4th ventricle as motor; the sensory fibres of the vagus and glossopharyngeal arising from the *fasciculus solitarius*, *fasciculus communis* and the large sensory nucleus in the dorsal folds of the medulla oblongata. Moreover the functions of the vagus nerve point clearly to the conclusion that this nerve is very largely if not mainly an efferent nerve rather than an afferent and that therefore in its centre of origin, including in this centre that of the medullary fibres of the spinal accessory, motor nerve cells must exist in sufficient quantity to give origin not only to the motor nerves of the striated muscles of the larynx, pharynx and œsophagus, but also to the motor nerves of the unstriated muscles of the alimentary tract.

Now a comparison of the function of the vagus with that of the abdominal splanchnics shows that a great split has taken place in the centres of origin of the katabolic and anabolic nerves of the same

[1] *Zoolog. Anzeig.* No. 227, 1886.
[2] *Archiv. f. Anat. u. Physiol.* Anat. Abtheil 1887, p. 368.
[3] *Journ. of Morphol.*, Vol. II. p. 51.

muscular tissue of the alimentary canal; in the vagus we find the great mass of motor fibres, in the splanchnics the great mass of inhibitory fibres. If therefore a difference exists in the nerve cells which give origin to the motor and inhibitory fibres of these muscles, that difference ought to manifest itself in these two regions and we ought to find that the solitary cells of the base of the posterior horn increase largely when the vagus nucleus is reached, just as the cells of Clarke's column increase at the origin of the abdominal splanchnic nerves.

Judging from the description given by Schwalbe[1] and from what I myself have observed in the case of the spinal cord of the pigeon, there can be no doubt but that the formation of the vagus nucleus is associated with a large increase of cells at the base of the posterior horn. The point however which requires further investigation is the respective shares taken in that increase by the cells of Clarke's column and the solitary cells of the base of the posterior horn. As far as I know no such investigation has been as yet published, so that at present we are hardly justified in concluding that the split which has taken place in the nerves of opposite function which govern the muscles of the alimentary tract has its counterpart in the central nervous system in the shape of a corresponding separation of the centres of those nerves, i.e. of the cell groups of Clarke's column and the solitary cells of Schwalbe respectively. At present all we can say is that taking these two sets of cells together we may look for the origin of both motor and inhibitory nerves of the alimentary tract in them, and that it is possible that further investigation will prove the truth of the suggestion here made, viz. that the solitary cells of the base of the posterior horn give origin to the motor fibres of the muscles of the alimentary canal while some of those of Clarke's column are the centres of the inhibitory fibres of the same muscles.

Cells of lateral horn.

In my former paper I spoke somewhat vaguely as to the meaning of these cells, because I did not sufficiently realize that the term lateral horn does not mean quite the same thing in different parts of the cord. Thus the lateral horn of the cervical region is not necessarily the same as that of the thoracic region either in structure, position or function. In the thoracic region the lateral horn or intermedio-lateral tract is in the case of the dog situated somewhat on the dorsal side of the middle line of the cord and is well pronounced; in the cervical region any

[1] *Op. cit.* See *x* in Fig. 362, p. 600.

projection that may be defined as a lateral horn is on the ventral side of the middle line in close proximity to the cells of the anterior horn.

This intermedio-lateral tract is according to Schwalbe most distinct in the upper part of the thoracic region, disappears gradually at the lower limit of the thoracic region and is absorbed into the cervical swelling, reappearing again as a distinct part of the anterior horn in the upper cervical region.

It is therefore conspicuous only in the thoracic region and contains there a well-marked group of nerve cells (E in Fig. 1) which as seen in the figure are in close connection with the solitary cells of the base of the posterior horn and the cells of Clarke's column. This group of cells is situated on the dorsal side of the intermedio-lateral tract. In addition to these cells some of the sections of the thoracic cord in man show on the ventral side of the tract (B in Fig. 1) the occurrence of other cells which are markedly larger, resembling much more the cells of the anterior horn, and which are very few in the thoracic region in comparison with the large number of the smaller cells already described. These large cells on the ventral side of the intermedio-lateral tract appear to me to form a connecting link between the somatic area of the anterior horn and the splanchnic area around the central canal.

It is this part of the lateral horn group of cells which forms, as I believe, the whole or greater part of the lateral group of cells in the cervical region; it is this group therefore which in all probability gives origin to the phrenic nerves, to some if not all of the large motor fibres of the spinal accessory; it is the continuation of this group which forms the *nucleus ambiguus* or so-called motor nucleus of the vagus and glosso-pharyngeal, which forms the nucleus of the facial and the motor nucleus of the trigeminal.

In this group of nerve cells we have in my opinion the origin of the motor nerve fibres belonging to the non-ganglionated group of splanchnic nerves—motor nerves which supply voluntary muscles derived not from the muscle plates but from the lateral plates of mesoblast—structures which segmented in the region of the head give rise to the voluntary muscles of the jaws and the gills but unsegmented in the trunk give rise to the diaphragm and transversus abdominis muscles.

What then is the function of the smaller cells which form the main portion of the intermedio-lateral tract in the thoracic region? Although we have no direct evidence to go upon I venture to suggest that these nerve cells give origin to the katabolic or motor nerves of the vascular and glandular systems, for in contradistinction to the cells of

Clarke's column they are most numerous in the upper part of the thoracic region and are therefore concerned in the formation of the cervical splanchnic nerves as well as of the abdominal splanchnics; they correspond in fact closely to the distribution of the motor nerves of the heart and blood vessels as given by me in my former paper. Again it is a significant fact that in the medulla oblongata a group of nerve cells exists known by the name of the antero-lateral nucleus, which is an isolated clump of nerve cells apparently belonging to the lateral horn, separate from the motor nuclei of the IXth, Xth and XIth nerves; as far as is known this group of nerve cells does not give origin to any outgoing nerve fibres of that region; the only function which has been suggested for it is based upon the experiments of Owsjannikow and Dittmar in Ludwig's laboratory, by means of which it was shown that the vasomotor centre in the medulla oblongata was confined to the position of this antero-lateral nucleus. The experiments also of Miescher in the same laboratory render it highly probable that the nerve fibres which spring from this group of nerve cells pass along the cervical region of the cord in the lateral columns in order to be distributed in the thoracic outflow of visceral nerves to the blood vessels which they supply. I am inclined to look on this antero-lateral nucleus as serially homologous with the smaller cells of the intermedio-lateral tract in the thoracic region and to consider this chain of nerve nuclei as giving origin to the motor or katabolic nerves of the vascular system.

I have ventured to put towards these suggestions of the meaning of these different groups of nerve cells in the hope that they may prove of assistance to others who are endeavouring to bring into harmony the relations between the structure and functions of the spinal cord. The arguments which I have used are I think, as far as they go, good; but I am ready to confess that they do not go very far. For the purposes of this paper it is a matter of no great importance whether the division of function of these four groups of cells—viz. those of Clarke's column, the solitary cells of the base of the posterior horn, and the two groups of the lateral horn—corresponds to the suggestions above enunciated; the important point is that these four groups of cells taken together give origin to the splanchnic efferent fibres, both ganglionated and non-ganglionated, that therefore the centres of the efferent nerves both somatic and splanchnic fall into two separate groups, of which the latter is situated in the neighbourhood of the central canal while the former is confined to the outlying anterior horn. If then the cranial nerves are

comparable to the spinal, we must find in the cranial region groups of nerve cells serially homologous with these splanchnic and somatic efferent spinal centres.

Definition of a complete segmental nerve.

Turning our attention now to the arrangement of the fibres composing a complete segmental spinal nerve, the results arrived at in my previous paper show that we must look upon both afferent and efferent roots as ganglionated, for the whole argument in my former paper as to the meaning of the sympathetic system was to prove that the sympathetic ganglia are the ganglia belonging mainly to the fine medullated fibres of the anterior roots, so that we must look upon a spinal nerve as possessing efferent or motor ganglia as well as afferent or sensory ganglia. These efferent ganglia have according to the observations of Onodi travelled away from the original ganglionic mass situated on the roots of the spinal nerve, so that we may term these motor ganglia "vagrant" in contradistinction to the "stationary" sensory ganglia on the posterior root; it follows then that a spinal nerve must be defined as formed by

1. A posterior root composed of afferent fibres, both somatic and splanchnic, the ganglion of which root is stationary in position and is always situated near the entrance of the fibres into the central nervous system.

2. An anterior root composed of (1) efferent non-ganglionated splanchnic and somatic fibres, and (2) efferent ganglionated splanchnic fibres characterised by the fineness of their calibre, the ganglion of which is vagrant and has travelled to a variable distance from the central nervous system.

Also it must be remembered that the anterior root fibres need not necessarily leave on one side the ganglion of the posterior root, for as pointed out previously both anterior and posterior roots pass into the spinal ganglion in the case of all the thoracic nerves of such an animal as the tortoise and also of the first two cervical nerves of the dog.

The essential characteristics therefore of a segmental spinal nerve are as follows:

1. Afferent nerve fibres, splanchnic and somatic, both in connection with a ganglion situated near the exit of the nerve from the central nervous system.

2. Efferent fibres which may either pass through the afferent

ganglion or may pass on one side of it and which are divisible into large fibred and small fibred groups, the latter of which is connected with a vagrant ganglion.

3. The efferent fibres, both somatic and splanchnic, may be traced to certain definite groups of nerve cells in the spinal cord; but no such groups are known in connection with the afferent fibres.

The cranial segmental nerves.

Having thus defined the nature of a fully formed spinal segmental nerve, we are able now to discuss more fully than in my previous paper the nature of the cranial nerves and see whether or no they conform in structure, origin, and function to the above definition of a complete segmental nerve. I propose to consider the cranial nerves seriatim, and seeing that the optic and olfactory nerves clearly do not conform to the above type I will commence with the consideration conjointly of the oculomotor and trochlear nerves[1].

Nerves of mid-brain, IIIrd and IVth cranial nerves.

The functions of the oculomotor nerve are well defined, it supplies a number of somatic muscles of the eye with motor fibres and it is also the motor nerve for the sphincter muscle of the iris and for the ciliary muscle; it is therefore purely an efferent nerve in existing animals, whatever it may have been originally.

It arises from a large number of rootlets which unite to form a single nerve trunk within the cranium; section of the nerve here shows that it is composed of both large and fine medullated fibres; the large fibres varying in size from 14·4—18 μ while groups of fine fibres varying in size from about 3—5 μ are to be found strongly resembling the groups found in the anterior roots of the thoracic nerves; in fact the structure of the nerve strongly resembles a typical anterior root. By means of a series of sections it is easy to see how the large fibres pass off to supply the eye muscles belonging to this nerve and how as we approach the oculomotor ganglion the small fibres separate out from the fibres forming the nerve supplying the internal rectus muscle and pass off as a separate group, forming the radix brevis, into the oculomotor ganglion.

[1] In order to compare these observations with those of my former paper, all measurements of size of fibres are taken from the dog unless expressly stated otherwise.

Here they enter into a large number of ganglion cells resembling those of a spinal ganglion and issue forth to form the short ciliary nerves, i.e. the motor nerves of the sphincter and ciliary muscles.

Fig. 1, Pl. XVI, is a small portion of one of a series of sections taken through an osmic preparation of the IIIrd nerve of the dog. The sections were cut from the roots of the nerve towards the periphery and the section of which Fig. 1 represents a part was through the nerve just before the oculomotor ganglion was reached. Great numbers of the smallest fibres are seen coming to the periphery of the nerve, preparatory to entering into the oculomotor ganglion. Soon after this section the cells of the oculomotor ganglion begin to appear and Fig. 2, Pl. XVI, represents a portion of one of the sections through the middle of the ganglion; all the fibres in among the ganglion cells are of the smallest size to be found in the IIIrd nerve. The continuation of the series of sections shows the formation of the short ciliary nerves from the ganglion, and as is seen in Fig. 3, Pl. XVI, the fibres of which these nerves are composed are all very small and very uniform in size.

In this way by far the largest portion of the ganglion is formed; in addition however to the mass of fibres coming from the IIIrd nerve, one or two fine nerves are found entering into the ganglion which come apparently from the naso-ciliary branch of the Vth and from the so-called sympathetic system. These small nerves considered as a whole are made up of very fine medullated fibres, of non-medullated fibres and of larger medullated fibres; all their large fibres pass away into the muscular branches of the oculomotor nerve and do not enter the ganglion, while the rest of the fibres pass into the ganglion and lose themselves in a small group of cells which appear to me to resemble in form and appearance those of the sympathetic system rather than spinal ganglion cells.

Neither part of this ganglion then is connected with any large fibred sensory nerves from the Vth, it is connected only with very fine efferent nerve fibres which come mainly from the IIIrd nerve; it is in fact as typical a motor vagrant ganglion as any in connection with the spinal nerves. We see then that Schwalbe is right in looking upon this ganglion as essentially belonging to the IIIrd nerve; that van Wijhe would have been right in considering it as wholly sympathetic if he had only known the true meaning of a sympathetic ganglion. Those observers are wrong who have endeavoured to force the IIIrd nerve into the position of a segmental nerve by making this ganglion into a posterior root ganglion. This

confusion has doubtless arisen as Gegenbauer[1] has pointed out by mixing up that part of the Gasserian ganglion, which forms the ganglion of the *ram. ophthalm. profundus*, with the oculomotor ganglion. In a recent paper by Beard[2] the distinction between the ganglion belonging to the Vth (meso-cephalic ganglion, Beard) and the true oculomotor ganglion has been pointed out.

It may be argued that the structure of the nerve cells points to a close relationship of the main portion of this ganglion with a spinal ganglion; this by no means necessarily follows, for the difference between a spinal ganglion cell and a so-called sympathetic ganglion cell depends probably upon the structure and number of the nerve fibres which enter and pass from it rather than upon their function; thus in the spinal ganglion the unipolar and bipolar cell is found associated with fibres which are medullated in both directions while the multipolar cell belonging to the efferent nerves of the vascular and glandular systems, i.e. the so-called sympathetic nerve cell, is according to the arguments given by me in my previous paper associated with a medullated fibre only in one direction, the other processes giving rise to a number of non-medullated fibres. The reason then why the nerve cells with which the fine fibred motor nerves of the sphincter and ciliary muscles are connected are of the spinal type is because the fibres do not lose their medullary sheath in their passage through the ganglion; as is seen in Fig. 3, Pl. XVI, the short ciliary nerves are medullated as much as the small fibres in the roots of the oculomotor nerve which give origin to them.

This then is in my opinion the reason why the cells of this vagrant efferent ganglion resemble the cells of a spinal ganglion, and it is a matter of great interest to inquire why some motor nerve fibres lose their medullary sheath in their passage through an efferent ganglion while others do not; it would lead too far away from the main purpose of this paper if I were to enter into this subject here, so I will simply suggest that the peculiarity of the sphincter and ciliary muscles consists in this:—that their action is to a certain extent voluntary and that in certain animals, e.g. birds, they are composed of striated muscle fibres; I hope however in a separate paper to treat especially of the relation which subsists between the structure and function of different muscles and their nerve supply.

[1] *Morphol. Jahrbuch*, 1887, p. 1.
[2] *Anatom. Anzieger*, 1887, p. 565.

Up to this point we can say that the oculomotor nerve conforms to the type of a spinal segmental nerve in that its efferent fibres are composed of a large fibred and a small fibred group of which the latter is in connection with a vagrant efferent ganglion.

Turning our attention now to the IVth nerve we see that its structure is very similar to that of the IIIrd; here also the nerve is efferent in function and is divisible into a large fibred and small fibred part, the large fibres supply the sup. obliq. muscle and vary in size from 14·4—18 μ. The small fibres which are more or less grouped together in the roots of the nerve vary in size from 3·6—5·4 μ, but as yet their destination is unknown; what structures they supply or whether they are in connection with any definite group of efferent ganglion cells requires a more special investigation of the nerve than I have as yet been able to give. We can however say this much that the IVth nerve so closely resembles in its structure the IIIrd as to allow the assertion that it too conforms to the type of the efferent portion of a segmental spinal nerve.

Where then are the afferent fibres of these two nerves? In the various schemes put forward by morphologists to explain the peculiarities of the cranial nerves some portion of the Vth nerve has generally been dislocated from the rest and forced to do duty as the afferent root of the IIIrd and IVth cranial nerves; attempts have been made for instance to look upon the *ram. ophthalmic. profund.* as a separate nerve which is the separated off posterior root of the IIIrd nerve; the history of such attempts together with a criticism upon them is given fully in Gegenbauer's[1] paper and need not detain us here. In my opinion no such supposition is necessary, for both these nerves possess within themselves structures which appear to me to have been originally the nerve cells and nerve fibres corresponding to the cells and nerve fibres of the stationary ganglion on a posterior root of a spinal nerve; so that in the possession of afferent fibres with a stationary ganglion as well as in the possession of efferent fibres these two nerves conform each to the type of a complete segmental nerve.

These structures I have found in their greatest perfection in the nerves of man; and the following description refers to osmic acid preparations of the cranial nerves of a woman aged 29 who died of perforation of the intestines. The roots of the nerves were taken out 24 hours after death with portions of the brain attached to them and

[1] *Op. cit.*

immediately placed in osmic acid 1 °/₀ solution. Each nerve was cut into a series of sections from its exit at the brain for some distance peripheralwards.

In Pl. XVII, Fig. 2, I give three sections of the IVth nerve selected out of the whole series. The section (2*a*) is through the root of the nerve close to its exit from the valve of Vieussens, and it shows that the nerve fibres are surrounded by a thick sheath of peculiar connective-tissue-like material; while section (2*c*) taken more towards the periphery shows that this sheath of peculiar tissue no longer exists. An examination of the sections between 2*a* and 2*c* shows that the manner of disappearance of this thickened sheath is very peculiar, it does not remain on the outside of the nerve fibres but forms roundish masses of the same peculiar material in between the nerve fibres themselves as is seen in (2*b*), so that the sections with a low power possess a remarkable resemblance to a section through a ganglion. At the peripheral side of this ganglion like formation these round masses of fibrillar-like material cease somewhat abruptly and the sections appear as in 2*c*.

Again in the IIIrd nerve the same formation is seen; here we have a nerve composed of a great number of rootlets and the sections show that the ganglion-like structure makes its appearance on the separate rootlets before they join together to form the conjoint root. Very striking indeed is the sight of a number of these rootlets each presenting in a greater or less degree the appearance represented in Fig. 1, Pl. XVII. Here too we see another point of resemblance between this structure and a spinal ganglion. In the latter it is of common occurrence to find that the formation of the ganglion has thrown many nerve fibres out of their previously parallel course, so that on transverse section of the ganglion many fibres are cut in a more or less longitudinal direction; the same deviation is found, as is seen in Fig. 1, in many of the large fibres of the IIIrd nerve, which are thrown out of their direct course in consequence of the formation of these rounded fibrillar masses in a way precisely similar to what occurs in the spinal ganglion. In fact if we imagined the living protoplasm of both the cells and the sensory fibres of such a spinal ganglion as that found in the thoracic region of the tortoise to have degenerated and disappeared so that only the envelope of these structures, so to speak, was left, we should have an appearance very like what is seen in the IIIrd and IVth nerves of man; the intact fibres of the anterior root in the tortoise, which as already mentioned do not in this animal run separately from the

ganglion, would correspond to the intact motor fibres of the IIIrd and IVth nerves in man.

When I first observed this peculiarity of structure in the roots of certain of the cranial nerves I imagined that the observation was entirely new; it was however pointed out to me later on that a paper had been written on the subject by Thomsen[1] working with Westphal; from his figures and description he evidently has seen the same structures which I have described, and although he does not recognize anything of the nature of a ganglion in the roots of such a nerve as the IIIrd yet he says that he has convinced himself that this fibrillar material is of phylogenetic importance and represents the remains of nerve cells; further that in the new-born infant he has found ganglion cells in connection with these circumscribed spots. If then we put together the observations of Thomsen which led him to the conclusion that these fibrillar masses were the remains of isolated nerve cells and mine which have shown that such masses are not isolated but are arranged just like the cells of a spinal ganglion, we must I think come to the conclusion that in these strands and clumps of fibrillar tissue found in the IIIrd and IVth nerves we have the remnants of nerve fibres and nerve cells which in by-gone animals were once of functional importance, but which have become useless and so lost their living protoplasmic contents, leaving the mere husk to show us still that these two nerves originally possessed nerve fibres and groups of ganglion cells corresponding in position and doubtless also in function with the nerve fibres and nerve cells of the stationary ganglia on the afferent root of a spinal nerve.

Any arrangement then of cranial nerves according to cranial segments must in the future reckon the IIIrd and IVth cranial nerves as completely or rather almost completely formed segmental nerves.

I say almost completely because in one respect only they do not apparently conform to the complete segmental type, viz. the large medullated fibres of their efferent roots supply muscles which are all somatic, being formed according to van Wijhe from the muscle plates of the first two cranial somites; they ought in addition to possess large medullated motor nerve fibres belonging to the non-ganglionated splanchnic group. Clearly such fibres do not exist in the nerves themselves, it however by no means follows that such fibres do not

[1] Virchow's *Archiv*, Vol. cix.

spring from centres of origin belonging to the same metamers as those of the IIIrd and IVth nerves but pass out at a different level, for it cannot be too often insisted upon that the position of the cell groups in connection with the nerve fibres is the true criterion of what forms a nervous metamer rather than the position of the exits of the nerve fibres. When I come to the consideration of the structure of the VIIth nerve I will deal with the evidence for the existence of non-ganglionated splanchnic efferent nerves belonging to the same metamers as the IIIrd and IVth cranial nerves (see p. 175).

Finally before passing on to the consideration of the other cranial nerves it is perhaps worth while to indicate how my view of the meaning of these two nerves differs from the views generally held as put forward by Gegenbauer in his recent paper. According to v. Wijhe both IIIrd and IVth cranial nerves are the ventral roots of two segmental nerves of which the *ram. ophthalm. profundus* and the rest of the Vth nerve form the respective dorsal roots. Against this Gegenbauer urges (1) that the *ram. ophthalm. profundus* bears no trace of being a separate nerve, it is only a branch of the Vth and therefore will not do for the dorsal root of the nerve of the 1st somite, and (2) that the IVth nerve is something separate and possesses sensory branches both in Amphibians and Selachians. The view which I put forward is this, both IIIrd and IVth nerves are in themselves complete segmental nerves of the type which Balfour[1] supposes to have been the original type "when mixed motor and sensory posterior roots were the only roots present"; that then owing to some change which occurred during the past history of the vertebrate the sensory parts of these two nerves degenerated and their place was taken by the sensory elements of the Vth nerve which, arising from segments behind, supplied those parts which have replaced the original sensory surfaces. Thus the *ram. ophthalm. profundus* is not a separate dorsal root but is a part of an extraordinary group of nerves, viz. those constituting the sensory root of the Vth nerve, which as we shall see have come up from hinder segments for the purpose of replacing the lost sensory nerve roots of a series of primarily fully formed segmental nerves.

These two nerves then form the primitive segmental nerves of the 1st and 2nd segments.

[1] *Comp. Embryol.*, Vol. II. p. 380.

Nerves of the hind-brain, Vth and VIth cranial nerves.

These two nerves are in reality made up of 3 nerves, viz. two efferent nerves and one afferent; the function of the VIth nerve is purely motor, it supplies only one muscle, *m. externus rectus*,—a muscle belonging to the somatic system being derived according to v. Wijhe from the muscle plate of the 3rd somite; the structure of the VIth nerve shows that it is composed of large medullated fibres varying in size from $14{\cdot}4\mu$—18μ together with a few smaller ones, but there is no sign of any distinct group of small fibres as in the IIIrd and IVth nerves.

The function of the so-called motor part of the Vth nerve is also very largely motor but it in all probability contains also fibres for the lachrymal gland as well as fibres which dilate the pupil and relax the ciliary muscle and possibly also vaso-dilator fibres; in accordance with its function the structure of the nerve is composed of large medullated fibres and of groups of small fibres which are doubtless in connection with the various vagrant ganglia such as the spheno-palatine found in connection with this nerve.

The muscles which are supplied by the large fibres of the motor part of the Vth nerve are derived from the ventral group of mesoderm segments according to v. Wijhe, i.e. from the lateral plates of mesoblast, so that these fibres belong to the group of non-ganglionated splanchnic efferent nerves and it follows that the motor part of V contains no somatic elements; if then we take together the two nerves VI with its somatic fibres and the motor part of V with its splanchnic fibres we obtain all the elements necessary for the construction of a complete spinal nerve as far as the efferent fibres are concerned.

If we ask next, Where are the afferent fibres with their root ganglion belonging to this nerve? the natural answer would be in the sensory root of the Vth with the Gasserian ganglion; and yet I venture to think such answer would be wrong, for the roots of the motor part of the Vth contain within themselves the remains of nerve fibres and ganglia of precisely the same character and in the same position as those already described on the roots of the IIIrd and IVth nerves. In the VIth nerve I have not found any sign of a regular ganglion formation, only a few scattered round masses of the same fibrillar material which are apparently the remains of isolated ganglion cells; I conclude therefore that primitively the motor part of V and the VIth nerve taken together form a complete segmental nerve which does not require the sensory

part of V to make it complete. It is possible that some portion of the sensory part of V which is in close connection with the origin of the motor part may belong to this segment. All that I desire to emphasise is the independence of the ascending root of V from this segment. In order to understand the meaning of the sensory root of V with its Gasserian ganglion, it will be better to consider it with the nerves of the medulla oblongata rather than in the present place; it will suffice here to say that the nerve fibres and ganglion cells of this root are all functional, there is no sign here of any degenerated ganglion cells or nerve fibres.

The primitive segmental nerve then of the 3rd or mandibular segment is represented by the 3rd branch or motor part of the Vth nerve together with the VIth nerve and the *ramus maxillaris superior* of the Vth nerve, i.e. the 2nd branch of the Vth has replaced the lost sensory elements of the original nerve just as the *ram. ophthalmicus* has replaced those of the two first segments. This is in accordance with the observations of Marshall and Spencer and v. Wijhe who all, as quoted by Gegenbauer, look upon the *ram. maxillaris sup.* as arising secondarily to the *ram. mandibularis.* As to the position of the VIth nerve the main fact is that it supplies the *m. rectus externus* which is derived from the 3rd somite; it is however by no means so clear that it therefore belongs to the 2nd ventral segment or hyoid segment rather than to the 1st or mandibular, for neither Marshall's nor v. Wijhe's arguments are sufficient to convince Gegenbauer that the VIth is the anterior root of the VIIth. The investigations of Ahlborn seem to show that in Petromyzon this nerve is much more closely connected with the Vth than with the VIIth; and the investigations of v. Wijhe show that the 1st gill-pouch is situated mainly under the 3rd somite, its anterior portion only lying under the posterior portion of the 2nd somite. In order to make his dorsal and ventral segments fit each other, and because he considers that the 2nd somite belongs to the mandibular segment, v. Wijhe is obliged to suppose that the hyoid segment is really double and that the ventral portion corresponding to the 3rd somite is not developed. Gegenbauer has however shown that such an assumption is not warranted; we have no reason to suppose that the hyoid segment is double and v. Wijhe's difficulty no longer exists if the VIth nerve belongs in reality to the mandibular rather than to the hyoid segment.

Nerves of the hind-brain, VIIth and VIIIth nerves.

In the VIIth nerve we find again an essentially motor nerve as far as function is concerned, for it supplies the muscles of the face; in addition it supplies efferent fibres to glandular structures, dilator nerves to blood-vessels by means of its well known branches chorda tympani &c. Like the motor part of the Vth nerve it is essentially a splanchnic efferent nerve, for the striated muscles it supplies all belong to the visceral group of muscles, being derived according to v. Wijhe from the ventral mesoderm segments, i.e. from the lateral plates of mesoblast. Examination of its structure shows again a division into large fibred and small fibred groups of nerves; the larger fibres passing off as the motor nerves of the facial muscles, while the fine fibres in the main separate out to enter into the geniculate ganglion and from thence to pass to their destination in the *chorda tympani, petrosus superficialis major* &c.

In man it can be very plainly seen that the majority of these fine fibres do not belong to the roots of the facial proper, but compose a series of rootlets situated between the facial and the auditory and known by the name of the *n. intermedius*. The contrast between the structure of the *n. intermedius* and that of the facial roots proper is very striking, and it is worthy of mention that although the *n. intermedius* is essentially a small fibred formation a few very much larger fibres stand out conspicuously among the smaller ones. I draw attention to this fact simply because it is possible that the further investigation of these larger fibres may throw light on the vexed question of the existence of nerves of taste in connection with the VIIth nerve. In neither dog nor rabbit can a distinct *n. intermedius* be spoken of; in both cases however, when the sections of the rootlets of the VIIth nerve are examined close to the exit of the nerve from the brain substance, it is found that many of the separate bundles are composed entirely or nearly entirely of the smallest fibres, of fibres in the case of the dog varying in size from about 3μ—4μ.

These bundles have a strong tendency to be grouped together apart from the rootlets containing the large fibres of the VIIth nerve; such bundles represent I imagine the *n. intermedius* of human anatomy and their fibres pass directly into the geniculate ganglion. Undoubtedly the cells of the geniculate ganglion are mainly connected with these small fibred groups and equally undoubtedly such nerves as the *chorda tympani* and *petros. superficialis major* which are known to be largely efferent in

function spring from this ganglion, so that we have very good grounds for looking upon this ganglion as a vagrant efferent ganglion of the same kind as the oculomotor ganglion. It may however be more than this, for I have not been able as yet to convince myself that it is free from connection with any group of large fibres as is the case in the latter ganglion. It is difficult to obtain a really good series through this ganglion, owing to the great difficulty of combining really good osmic staining with freedom from splinters of bone, so that I can only say that my present sections point to the conclusion that this ganglion is not wholly efferent, for it appears to be in connection with a small group of large fibres which are probably afferent in nature. Whether this group if it exists comes from the *n. intermedius* or not I cannot at present say; it is one of the numerous points of interest in connection with the relation between the size of a nerve fibre and its function which requires investigation. One point however is worthy of mention, viz., that according to the universal agreement of morphologists, the VIIth nerve in the lower vertebrates possesses a ganglion which is in connection with sensory nerves; in man, and in the higher vertebrates, it would appear that the region supplied by such sensory nerves is innervated by the Vth and not by the VIIth, so that, considering the intimate relationship which is known to exist between the VIIth and Vth in these lowest classes of vertebrates, it is possible that a remnant of sensory fibres should still exist in the VIIth nerve of the higher vertebrates which would belong to the same system as the sensory fibres of the Vth nerve, and therefore as we shall see later on to the system of ascending roots.

The large fibred rootlets of the VIIth nerve contain a few fibres of the smallest size, but are mostly composed of the motor fibres of the facial muscles, which are very uniform in size and measure about $10{\cdot}8\mu$. In addition to these there are also a very large quantity of fibres which measure between 7μ and 8μ.

The evidence then of the structure and function of the VIIth nerve shows that it corresponds to a complete segmental nerve, in that its efferent root contains a large fibred non-ganglionated splanchnic portion and a small fibred ganglionated splanchnic portion with its vagrant ganglia in the shape of the geniculate ganglion, submaxillary ganglion, &c.

As far as its stationary ganglion belonging to its afferent root is concerned we find here, as in the other motor cranial nerves already considered, evidence of a degenerated ganglionic structure close to the

exit of the nerve from the brain. In Fig. 3, Pl. XVII, I give a picture of a section through this primitive ganglion of the VIIth nerve. In my opinion, then, the VIIth nerve is similar to the IIIrd, IVth and *pars mandibularis* of the Vth in that it has lost its primitive stationary ganglion and the afferent fibres in connection with that ganglion; I would therefore look upon the existing sensory distribution of the nerve as belonging to the same system as that of the Vth, i.e., as already mentioned, a system of sensory nerves which has taken the place of the lost sensory elements of the primitive group of segmental nerves.

One component of a fully formed segmental nerve still remains for consideration, viz. the somatic efferent portion. In my previous paper[1] I called attention to the remarks of Schwalbe, that many observers considered that some part of the fibres of the VIIth arose from the nucleus of the VIth nerve, and also that the *m. orbicularis palpebrarum* and *m. frontalis* often remain unaffected in cases of bulbar paralysis when the nucleus of the VIIth nerve has been destroyed by disease. I imagined then that possibly these muscles might be supplied by fibres from the nucleus of the VIth nerve.

Now I had noticed that the motor fibres supplying the somatic muscles of the eye were very much larger than those supplying the facial, pharyngeal and laryngeal muscles, i.e. in these cases a distinct difference in size, between somatic motor and splanchnic motor non-ganglionated nerve fibres, the former varying from $14{\cdot}4\mu$—18μ, the latter from 9μ—$10{\cdot}8\mu$. A similar difference in size of fibres is very evident in the formation of the phrenic nerve. In Fig. 5, Pl. XVI, I give a section through the 4th cervical nerve of the rabbit at the point where the fibres of the phrenic are separating out from the rest of the fibres of the 4th cervical nerve; as is seen the fibres of the phrenic nerve (B) are very uniform in size and much smaller than the large motor fibres of the somatic muscles supplied by the 4th cervical nerve (A). If then any somatic fibres from the nucleus of the VIth passed out in the VIIth nerve they ought to be conspicuous by their size among the smaller motor fibres of the VIIth. Reasoning in this way I was much struck to find that in the roots of the VIIth nerve of the dog a small group of large fibres stands out most conspicuously; the size of the fibres being the same as those of the large motor nerves of the eye muscles, viz. 16μ—18μ. In Fig. 4, Pl. XVI, these fibres are shown in the rootlets

[1] *Op. cit.* p. 68.

as they are found. Here then appeared to be positive evidence of the existence of somatic motor fibres in the roots of the VIIth nerve. Upon examination, however, of the nerves supplying the orbicularis and frontalis muscles I was unable to find that their fibres were different in size to those of the other facial muscles, and, indeed, section of the branches of the VIIth nerve after they had passed out of the stylomastoid foramen failed to show the presence of any group of especially large fibres.

There is then no evidence that any one of the muscles of expression is innervated differently from the rest, they all belong to the same group, they are all supplied by splanchnic non-ganglionated fibres. Yet the fact remains that certain muscles escape paralysis when the rest are paralysed by disease situated centrally. During the last year an explanation of this peculiarity has been given by the observations of Mendel[1], who found that the *m. orbicularis palpebrarum* was innervated by fibres which arose from the posterior portion of the oculomotor nucleus and travelling along the posterior longitudinal bundle passed out with the roots of the VIIth nerve to reach their destination. In fact in these muscles and their nerve supply we have the missing splanchnic non-ganglionated efferent portion of the oculomotor and trochlearis segments to which reference was made on p. 169.

Still firmly convinced in my own mind that these large fibres represented the somatic efferent elements of the facial nerve, I dissected out the whole facial from its origin to its exit from the stylomastoid foramen, stained it with osmic and cut sections of it at intervals, and found that at a certain point in the nerve all the very large fibres which were present in the nerve at that point came together to the periphery of the nerve, and forming a distinct though small nerve branch disappeared out of the series of sections. It is clear then that these large fibres have a very definite meaning, for they go off by themselves to form a separate nerve; I was unable to trace this slender nerve to its destination but it seemed to me at the time that the only muscle it could possibly supply was the *m. stapedius*, and as the rest of the very large fibres left the main trunk in one of the branches from the geniculate ganglion I imagined that they probably were intended for the supply of the *m. levator palati*, which according to all the text-books is innervated from the VIIth nerve. That such were the actual or probable destinations of these large fibres I have stated in the preliminary

[1] "Ueber den Kern-Ursprung des Augen-Facialis." *Neurolog. Centralblatt*, 1887, p. 537.

communication[1] to this paper; in both cases that statement is unfortunately erroneous, for in the first place I determined before the full paper was published to trace this fine nerve into the *m. stapedius*, and one of the students, Mr Waggett, kindly undertook to dissect out the facial nerve and the stapedius muscle and to make sections; this he has done and has proved to me conclusively that the large fibres do not supply the stapedius muscle and do not leave the main trunk until nearer the exit of the facial from the bone; as yet he has not determined their ultimate destination. I beg here to thank him for pointing out to me the erroneous conclusion to which I had come from my own imperfect observation. In the second place, the observations of Beevor[2] and Horsley which have recently been published show that the text-books of anatomy are wrong with respect to the *m. levator palati*, for that muscle is innervated by the spinal accessory nerve and not by the facial, so that the large fibres of the facial cannot supply that muscle.

At present, then, I must confess that I have no evidence to offer as to the existence of the somatic efferent nerves of the hyoid segment, and can only point out that v. Wijhe's 4th somite aborts and does not form any muscles, which, if v. Wijhe is wrong, as Gegenbauer thinks, in supposing that the hyoid segment is double, may mean that the somatic muscles of the hyoid segment have for some reason or other ceased to be developed. The other supposition would be that the VIth nerve is all that remains of the somatic efferent nerves of both the mandibular and hyoid segments.

As to the VIIIth nerve, I do not think it advisable at present to offer any definite opinion upon its position; for I do not as yet know sufficient about its structure or the origin of its nerve fibres to enable me to speak with any confidence with respect to it. I can therefore only repeat that it is not necessary to look upon it as part of the VIIth, and that it occupies a special position as a nerve of special sense, which may possibly justify its claim to an independent position. I am pleased to find that I am supported in this view by the observations of Osborn[3], who from his investigations into the brain of the Amphibia is inclined likewise to place the auditory nerve in a separate category.

Summing up the results of our investigation into the nature of the

[1] *Proceedings of the Roy. Soc.* Vol. XLIII. p. 389.
[2] *Proceedings of the Roy. Soc.* Vol. XLIV. p. 269.
[3] *Op. cit.* p. 71.

cranial nerves considered up to this point we see that they form a natural group, all the members of which possess the same characteristics.

In this group of motor cranial nerves formed by the IIIrd, IVth, VIth and motor part of Vth, and VIIth nerves we find at least 4 fully formed segmental nerves, which for some reason or other have lost a certain portion of their original components.

Nerves of the Medulla Oblongata.

In the next group of cranial nerves, viz. those which arise from the medulla oblongata, we again find all the components which make up a fully formed spinal nerve or rather group of nerves; here however there is no sign of any degeneration of any special group of fibres, but rather of a dislocation and scattering of the different components, so that the cranial nerves of this group form parts of a number of segmental nerves instead of each one forming a single nerve; thus the somatic efferent nerves are collected together in the hypoglossal nerve; the somatic afferent fibres of this region do not pass out with the nerves of the medulla oblongata, but help to form part of the sensory root of the Vth nerve, the only other somatic sensory nerves in the mammal being the auricular branches of the vagus; the splanchnic nerves, both afferent and efferent, form the glossopharyngeal, vagus and medullary portion of the spinal accessory. Taking then these nerves in order we must examine first the glossopharyngeal or IXth cranial nerve.

By the general consent of all morphologists this nerve holds a perfectly definite position among the cranial nerves; it is the nerve of the 1st branchial segment and supplies only the ventral portion of that segment; i.e. it is a purely splanchnic nerve and contains no somatic elements. Further, it is the foremost of a series of nerve roots, viz. those of the vagus and the medullary portion of the spinal accessory which are all built up on the same plan, are all splanchnic nerves and supply the ventral portions of the rest of the branchial segments. The nature of the fibres which form the nerves of this group has already been largely considered in my former paper, so that it is not necessary to dwell upon them here. I will however briefly recapitulate. The IXth and Xth each possess two ganglia, the *ganglion jugulare* and *ganglion petrosum* on the one hand, and the *ganglion jugu-*

lare and *ganglion trunci vagi* on the other[1]. In connection with the *ganglion petrosum glossopharyngei* we find efferent nerves such as those forming part of the *n. tympanicus* and supplying the parotid gland with both secretory and vaso-dilator fibres. In connection with the *ganglion trunci vagi* we find, as pointed out in my former paper, the motor fibres of the alimentary tract.

The structure of the roots of the IXth and Xth nerves resemble each other in the fact that they are composed mainly of very small fibres varying in size from 1·8 μ to 3·6 μ. They also contain large fibres the size of which does not exceed 10·8 μ. Such fibres however are very sparing in the glossopharyngeal root, but more numerous in the roots of the vagus and the medullary roots of the spinal accessory. With respect to the function of the different fibres we are met with the difficulty, that at present no criterion exists in the structure of a nerve fibre by which we can decide whether a nerve fibre of any particular size is afferent or efferent; we cannot therefore as yet decide by the mere inspection of sections of the roots of the IXth nerve the disputed point as to the existence in this root of nerve fibres which are motor to striated muscles. The large fibres may be motor to such muscles as the *m. stylo-pharyngeus* or they may be sensory fibres to some tract supplied by the nerve.

In the case of the Xth and XIth nerves it is easy to determine the size of the large motor nerve fibres of the laryngeal muscles by section of the vagus nerve at the place where the inf. laryngeal is just leaving the nerve, and it is very instructive to see how a number of the largest fibres in the nerve (about 10·8 μ in size) sort themselves out into a group at the periphery of the nerve trunk and so form the inf. laryngeal. These large fibres probably come partly from the medullary roots of the spinal accessory nerve, in which fibres of that size are freely found. Such medullary roots however merge imperceptibly into the roots of the vagus proper.

In accordance with the arguments used in my former paper and in this paper, I consider that the two jugular ganglia represent the stationary afferent ganglia of the IXth and Xth nerves, while the *ganglion petrosum glossopharyngei* and the *ganglion trunci vagi* represent their

[1] The roots of IX, of X, of the medullary portion of XI and that rootlet which springs from XI to pass over to XII and to form its ganglionated root, all have their fibres imbedded in a peculiar matrix somewhat resembling the degenerated material of the 1st group. This is however not arranged so as to form ganglia, and cannot be interpreted as in the first case.

vagrant efferent ganglia. In the higher animals the stationary ganglia are in connection almost entirely with splanchnic afferent fibres, for, with the exception perhaps of the auricular branch of the vagus, the somatic afferent nerves of this region have all passed away into the ascending root of the Vth nerve. In fishes, however, a marked sensory nerve, belonging I imagine to the somatic system, viz. the *r. lateralis*, arises from the vagus; and section of this nerve in the skate shows that it is composed mainly of large fibres which are in marked contrast to the small fibred structure of the *r. intestinalis*.

An interesting point in connection with the vagrant or efferent ganglion of the vagus is the fact that its nerve cells belong to the so-called spinal type of ganglion cell, and not to the so-called sympathetic type, although as I have already explained[1], the fine motor fibres of the alimentary tract lose their medullary sheath after passing through the ganglion. Here then apparently we have connected with ganglion cells motor fibres which are intermediate in type between the motor fibres of the iris muscles and those of the muscles of the vascular system; and I imagine that the reason why these nerve cells are of the spinal type is because, as in the case of the oculomotor ganglion already discussed, only one nerve fibre leaves the cell and only one enters it, that in fact the difference between the non-medullated fibres of the vagus trunk and those of the so-called sympathetic system may be explained by supposing that the multiplication of fibres takes place differently in the two cases: in the former the nerve fibre divides some time after it has left the cell, in the latter the division takes place in the cell itself. In osmic preparations of the vagus nerve near the diaphragm I have often thought that the outlines of the separate nerve fibres showed more distinctly than is usual in a mass of non-medullated fibres, and many of the students who attend my lectures to whom I have shown the specimens have thought that a very fine medullated sheath existed round each axis cylinder, so that the loss of medulla was not absolutely complete; I have not, however, been able to convince myself that such is the case, in the dog at all events, and I find that Ranvier[2] in the recently issued number of his Histology also looks upon the vagus as largely composed of non-medullated fibres. On the other hand, the great mass of fibres which leave the *ganglion trunci vagi* of the crocodile are medullated; and in connection respectively with the retention and loss of the motor

[1] *Op. cit.* p. 24.

[2] *Traité technique d'Histologie, 7th Fascic.* Paris, 1888, p. 1022.

functions of the nerve according as it was cut above or below the ganglion, it is to be noticed that in the former case most of the medullated fibres showed no signs of degeneration, while in the latter case the most noticeable feature was the marked degeneration of these fibres.

Seeing that, as already mentioned, I propose in a future paper to discuss the exact differences in size of fibre, loss of medulla and connection with ganglion cells which exist in the motor nerves of the same animal according to the nature of the muscle supplied I will not dwell longer on this point now. I would simply desire to impress upon my readers the necessity of recognising that the vagus is very largely if not mainly a motor or efferent nerve. It is the motor nerve not only of the laryngeal and pharyngeal muscles but also of the unstriped muscles of the bronchi, and of the muscles of the whole alimentary tract as far as the hind gut and its appendages. In accordance with its motor character we see that a large number of its fibres pass on one side of the *ganglion jugulare* and enter directly into the motor ganglion called the *ganglion trunci vagi.*

The Spinal accessory Nerve.

This was the nerve the consideration of which led me to the conception of the division of the nerve roots into the two great somatic and splanchnic divisions. It is clearly an efferent nerve, it is equally clearly, as I have already shown in my previous paper, mainly composed of two kinds of fibres, the one large in size, and the other very small; the large ones arise in all the roots of the nerve, the groups of small fibres are confined to the medullary and upper cervical roots and pass into the *ganglion trunci vagi.* In this nerve we have, then, clearly a separated group of motor fibres which take origin from the same metamers as the vagus and the upper cervical nerves. This group of motor nerves is clearly composed of a ganglionated splanchnic part, i.e. the small fibred portion and a non-ganglionated part; and the interesting question arises, Are these non-ganglionated large-fibred nerves splanchnic or somatic? According to the suggestions of my former paper the voluntary muscles supplied by this nerve belong to Charles Bell's system of respiratory muscles, and the nerve therefore is a purely splanchnic nerve. If this suggestion is a true one we have the very important fact that the efferent splanchnic fibres of the upper cervical region, both ganglionated and non-ganglionated, have separated out from the rest of

the components, with which they ought to be associated to form a complete nerve, and thus have formed a separate nerve root. It is necessary then to consider the position of the muscles supplied by this nerve. In the first place, we see that the large motor fibres of the medullary roots of this nerve, together with those of the vagus proper, supply the laryngeal and pharyngeal muscles; these are clearly splanchnic muscles, and not somatic[1]. The main bulk of large fibres supply the trapezius and sterno-cleido-mastoid muscles, of which the latter muscle is recognised as an isolated portion of the former; the question for discussion therefore resolves itself into this. Does the *m. trapezius* or *m. cucullaris*, as it is called in the lower animals, belong to the splanchnic or somatic muscular systems? For the answer to this question I am indebted to my friend Dr H. Gadow, whom I beg here to thank for this and other assistance. He tells me that the evidence of comparative anatomy distinctly points to the conclusion that the cucullaris group of muscles was originally a visceral or splanchnic group belonging to the same set of muscles as the mylohyoid, platysma, and perhaps the digastric. He argues that the original visceral muscles of the branchial region have been largely invaded and superseded by the ventral longitudinal somatic muscles, so that only portions of them have remained on each side of the median ventral muscles. These portions then have in their turn spread over the invading somatic muscles ventralwards and so formed the mylohyoid, platysma, trapezius and sterno-cleido-mastoid muscles. If this be the case then all the large fibres of the spinal accessory supply splanchnic muscles, so that this nerve is to be looked upon as the separated out splanchnic portion of the efferent roots of the upper cervical nerves, and contains all their splanchnic efferent fibres, both ganglionated and non-ganglionated, with the exception of those fibres which form the phrenic nerve.

The Hypoglossus Nerve.

It is unnecessary to add anything to my previous description of this nerve; it is a purely motor nerve, it is motor to the longitudinal ventral muscles, i.e. it is a somatic motor nerve. It clearly represents the separated somatic efferent nerves of this region, and cannot be

[1] In the pharyngeal muscles supplied by this nerve the levator veli palati ought to be included according to the observations of Horsley and Beevor; we have not at present sufficient evidence to enable us to judge of the position of this muscle.

looked upon as a separate segmental nerve any more than the anterior roots of the 1st or 2nd cervical nerves. As to the so-called ganglionated root of the XIIth nerve noticed by Froriep, no difficulty appears to me to arise. It is easily seen in the dog, and I have shown the structure of it in my former paper[1]. I have also in the figures on Pl. III. of the same paper indicated the nature of its origin; it is simply one of the rootlets of the medullary portion of the XIth nerve which runs with the XIIth nerve instead of with the Xth. Its presence no more indicates a posterior or afferent root to the XIIth nerve than the *ganglion trunci vagi*, and the small ganglia found on some of the other rootlets of the XIth nerve make this latter nerve an afferent as well as an efferent nerve.

Summing up, then the peculiarities of this second group of cranial nerves, viz. IX, X, XI and XII together with the sensory part of V, we see that they represent a primitive group of at least five segmental nerves which unlike those of Group I. have not lost any particular component, but have become broken up and split into various portions, doubtless in connection with the making good of those parts, the loss of which caused the degeneration of nerve structures observed in Group I.

This group of cranial nerves is further characterised by the extensive distribution of its peripheral nerves both somatic and splanchnic, for not only is the whole of the alimentary canal and its adjuncts with the exception of the hind gut and its appendages, innervated by the vagus nerve, but also in the nerve of the lateral line we have the somatic sensory elements of this group extending along the whole length of the body. Further, this group is characterised not only by the extensive distribution of its peripheral nerves but also by the extensive origin of its nerve fibres in the central nervous system, as will appear in the following section.

Origin of the Efferent Fibres of the Cranial Nerves.

As far as the origin of the cranial nerves is concerned I can add little to what is already known and described in the anatomical text-books, the substance of which was epitomised by me in my former paper; this is amply sufficient to show that the groups of cells which give origin to the cranial nerves belonging to the two groups treated of

[1] *Op. cit.* Pl. I. Fig. 5.

in this paper are the direct continuation of the corresponding cell groups found in the spinal region, that in fact the spinal nature of these cranial nerves is shown in the groups of cells from which their fibres arise just as clearly as in the correspondence between the structure and function of their peripheral nerve-fibres already treated of. In the first place, the centres of origin of the somatic efferent nerves are absolutely well-known in the groups of cells forming the nuclei of the IIIrd, IVth, VIth and XIIth nerves, which are in structure and position undoubtedly the continuation of the cell groups of the anterior horn. They show clearly the very significant fact, which is by no means confined to the cranial region, that this group of cells does not form a continuous column, but that the separate groupings point strongly to an original metameric arrangement of the centres of origin as well as of the peripheral distribution of these somatic nerves. It is worthy also of notice that the nuclei of the IIIrd and IVth nerves are so close together as to be continuous, while that of the VIth is some distance removed from them; as though the metamers supplied by III and IV were more closely related to each other than to that supplied by VI. Again, considering the smallness and compactness of the region supplied by the XIIth nerve the extent of its nucleus is most striking, for it extends along the whole length of the medulla oblongata; and considering that this nerve represents the somatic efferent part of the vagus nerve, i.e. of a nerve supplying a number of splanchnic segments, it is most probable that this extended nucleus of origin indicates a corresponding number of somatic centres which have coalesced to form one long nucleus.

In the description of the nuclei of origin of the cranial nerves given in the text-books of human anatomy, such as Schwalbe and Rauber, it is clearly pointed out and indeed has long been known that the cranial motor centres are divisible into two groups; the one the group already considered, and the other a lateral group corresponding to the lateral horn in the spinal region which gives origin to the motor nerves of the trigeminal, facial, vagus and spinal accessory. The morphological importance of this division was recognised and emphasised by Hill[1], who however failed to understand its true import as he had not read v. Wijhe's paper[2]. My work on the true meaning of the sympathetic system in combination with v. Wijhe's discovery that the muscles

[1] Plan of central nervous system. Cambridge, 1885.

[2] *Ueber die Mesoderm segmente u. die Entwickl. der Nerven des Selachierkopfes.* Amsterdam, 1882

supplied by these two sets of motor fibres arise from the dorsal and ventral mesoblastic segments respectively, enabled me to recognise the true meaning of this separation of the motor nuclei of the cranial nerves into two well-defined groups, of which the one gave origin to the somatic efferent nerves and the other to the splanchnic non-ganglionated efferent nerves. In his last paper His[1] has also begun to appreciate the importance of this separation of cranial motor nuclei into two groups; as however he has apparently not read my former paper he has failed to understand its full significance.

The centres then of the splanchnic non-ganglionated efferent nerves form the well defined group of cranial nuclei known as the motor nucleus of the trigeminal, the nucleus of the facial, the motor nucleus of the vagus group, and the nucleus of origin of the spinal accessory fibres. In connection with these nuclei various important questions arise which are of interest but which, since they are subsidiary to the main argument of this paper, it is not necessary to discuss here; such questions are the meaning of the upper nucleus of the trigeminal, the exact origin of the fibres of the facial which supply the *m. orbicularis palpebrarum*, and the limits of the spinal accessory nucleus. The main point is that the striated muscles which arise from the ventral mesoblastic segments, i.e. from the splanchnic segments, are innervated by nerves which not only leave the central nervous system separately from the nerves which innervate the muscles of the somatic segments, but also have in the central nervous system itself separate centres of origin from those latter nerves. These centres of origin are the continuation of the large celled groups in the lateral horn of the spinal region (see p. 160). Here too we find that these nuclei of origin are distinctly arranged metamerically, and it is worthy of notice that these metameric nuclei extend far into the cervical region, and by the formation of a long-ascending root give origin to a single nerve trunk, viz. the spinal accessory.

We come next to the origin of the splanchnic ganglionated efferent nerves of the cranial region, i.e. to the continuation of the groups of Clarke's column, the solitary cells of the base of the posterior horn, and the small cells of the intermedio-lateral tract. According to the conjectures already made we should not expect to find the small cells of the intermedio-lateral tract, i.e. the centres of katabolic nerves of the vascular and glandular systems, prolonged beyond the antero-

[1] *Archiv f. Anat. u. Physiol. Anat. Abtheil.* 1887, p. 379.

lateral nucleus in the medulla oblongata. Of the other two groups the nerve cells of Clarke's column are most easily followed, owing to their shape; they form part of the nucleus of the vagus and glosso-pharyngeal and terminate apparently in the large group of cells forming the *locus cæruleus.* These latter cells as pictured in Henle[1] resemble most closely the cells of Clarke's column.

With respect to the continuation cranialwards of the solitary cells of the base of the posterior horn I do not know of any observations which would enable us to follow them further than the vagus and glosso-pharyngeal nucleus; I have already given my reasons for believing that the vagus nucleus on the floor of the 4th ventricle (see Fig. 1. Pl. XVIII) is essentially a motor nucleus giving origin to the ganglionated motor fibres of the alimentary tract; and I feel that we cannot at present attempt to differentiate in the higher cranial regions between the origins of the ganglionated efferent anabolic and katabolic nerves or between the two contiguous groups of nerve cells which are known in the spinal cord as the cells of Clarke's column and the solitary cells of the posterior horn respectively. It is, however, possible to make certain suggestions for future investigations in this direction. Thus we find that, according to Henle[2], the descending root of the trigeminal nerve consists at its commencement of fine fibres only; soon however large fibres mix with them, which increase in number as the descending root approaches the nucleus of the trigeminus, until at last the root appears to consist almost entirely of large fibres; along the whole length of its course the root is accompanied by large bladder-shaped cells in strong contrast to the pigmented cells of the *locus cæruleus,* which latter are situated in close contiguity to it. If then, as is most probable, the observations of Henle and Forel that this descending root is composed of motor fibres are correct, it is possible that the three groups of nerve cells which give origin (1) to the small fibres of this root, (2) to the large fibres of this root, and (3) which form the cells of the *locus cæruleus* correspond to the three groups of nerve cells which in the spinal region give origin to the splanchnic ganglionated and non-ganglionated efferent fibres respectively. The nerve cells of the *locus cæruleus,* although strongly pigmented, are probably not entirely devoid of function, for according to Schwalbe[3] they are not pigmented in children or in mammalia generally.

The origin of the ganglionated small-fibred motor nerves of the

[1] *Op. cit.* p. 256. [2] *Op. cit.* p. 253.
[3] *Op. cit.* p. 678.

sphincter muscles of the iris and of the ciliary muscles is, as far as I can find out, not clearly known; Hensen and Voelcker's experiments seem to point to an origin for these nerves in front of and separate from the origin of the nerves which supply the striated muscles of the eye. The arrangement of the nerve fibres in the rootlets of the IIIrd nerve at their exit from the central nervous system appears to me to suggest a separate origin for these fibres, for certain of these very fine rootlets are almost entirely composed of the smallest fibres to be found in this nerve. This is especially noticeable in the case of the rabbit, and is strongly suggestive of the commencement of an intracranial separation of the small and large fibres. We are in fact reminded here of the same kind of separation as is seen clearly in the case of the VIIth nerve, where groups of the smallest fibres mixed with a few large ones separate out to form the *n. intermedius.*

The origin of this latter nerve is most interesting if the observations of Sapolini[1] are correct, for this observer asserts that the *n. intermedius* arises as a long ascending root of the same character as the ascending root of the vagus. If this be so we find then here at the border of the two groups of cranial nerves an ascending root composed largely of splanchnic ganglionated efferent fibres as the companion of the ascending root of the spinal accessory, which is composed mainly of splanchnic non-ganglionated efferent fibres.

The origin of the Afferent Fibres of the Cranial Nerves.

It is impossible to trace the centres of origin of the afferent fibres in the same way as we have been able to do for the efferent fibres, and to compare them directly with the corresponding centres in the spinal cord, because even in the spinal cord itself no well-defined groups of nerve cells exist with which it has been proved that afferent nerves have connection.

The two main characteristics of the spinal afferent fibres are the presence of the ganglion on the posterior root and the presence of the substantia gelatinosa Rolandi with its scattered small cells in the posterior horn. Of these two the former has already been considered, and it has been shown that the cranial nerves correspond to the spinal in this respect. As far as the substantia gelatinosa Rolandi is concerned this is found in the cranial nerves, as is well known, only in close con-

[1] Quoted by His.

nection with the ascending root of the Vth nerve. As to the degenerated nerve fibres of Group I. of the cranial nerves I have not attempted at present to trace them into the central nervous system. I can only say that the nerve cells of the *substantia nigra* appear from their position and from the direction of the long axis of many of their cells to have been originally connected with some of these nerve fibres belonging to the IIIrd nerve; I do not however think that such nerve fibres were afferent, for it is improbable that such a group of nerve cells as we find forming the *substantia nigra* should give origin to afferent nerve fibres. As, however, I propose to discuss the meaning of the cells of the *substantia nigra* later on I will not dwell further on this question here.

The afferent nerves of Group II. are most interesting in their distribution in the central nervous system, for not only are they distinctly separated into somatic and splanchnic groups but also both groups arise from the cervical region of the spinal cord as well as from the medulla oblongata. Thus all the sensory nerves of V forming the somatic afferent nerves arise from the ascending root of V, a bundle of fibres which has been traced as far as the middle of the cervical region; so also the ascending root of X or *fasciculus solitarius* or respiratory bundle formed by fibres of the IXth and Xth nerves is afferent in function, and, as has been already pointed out, contains if His is right all or nearly all of the afferent fibres of the latter nerve. This bundle of fibres has not been proved to arise from any groups of nerve cells in the medulla oblongata and has been traced into the spinal cord as far as the origin of the phrenic nerves.

Further, according to His[1] these afferent ascending roots of both the trigeminal and vagus nerves are in an embryo of 4½ weeks old quite superficial in position; so that we have evidence of a series of ascending roots in the cervical region of the cord of the same type as the spinal accessory. Including the spinal accessory itself these ascending roots respectively consist of (1) somatic afferent fibres, *i.e.* the ascending root of the trigeminal; (2) splanchnic afferent fibres, *i.e.* the ascending root of the vagus; (3) splanchnic non-ganglionated efferent fibres, *i.e.* the cervical portion of the spinal accessory; (4) splanchnic ganglionated efferent fibres, *i.e.* the *n. intermedius.* These form in fact a series of roots arising from spinal as well as cranial regions, which taken together contain all the elements of a complete segmental nerve or group of nerves with the exception of the somatic efferent

[1] *Op. cit.* p. 374.

fibres. Although these latter fibres do not form an ascending root yet they evidently are not missing from this system, for the whole argument of Gegenbauer, as already pointed out, goes to show that the hypoglossal nerve may be looked upon as the remnant of a system of somatic efferent roots which were spinal as well as cranial.

Summary.

In order to put clearly before my readers the results of the previous pages, I have endeavoured in Pl. XVIII to express in the form of diagrams the views therein expressed. In Fig. 1, Pl. XVIII, I have copied a section of the medulla oblongata from Edinger[1], and on the one half of the section I have lettered the groups of nerve cells *A*, *B*, *C*, *D*, *E*, to correspond with the lettering of the section of the spinal cord given in Fig. 1, p. 155. In both cases the letters have the same signification viz.

A (Cells of ant. horn)
= nucleus of efferent nerves to somatic muscles.

B (Large cells of lat. horn)
= nucleus of efferent nerves to striated splanchnic muscles.

C (Cells of Clarke's col.)
= nucleus of anabolic (inhibitory) nerves to splanchnic glandular system and to muscles of viscera and of vascular system.

D (Solitary cells of post. horn)
= nucleus of motor nerves to muscles of viscera.

E (Small cells of lat. horn)
= nucleus of katabolic (motor) nerves to splanchnic glandular system and to muscles of vascular system.

On the other half of the figure, I have lettered the groups of cells in the same way as Edinger has done and in addition have coloured the different groups of motor cells red, yellow, and green and the two tracts of sensory fibres blue and purple; the signification of the colours is as follows:—

Red (nucleus of XII) = somatic efferent.
Green (so-called motor nucleus of X, &c.) = splanchnic non-ganglionated efferent.
Yellow (part of nucleus of X and XI) = splanchnic ganglionated efferent.
Blue (ascending root of X) = splanchnic afferent.
Purple (ascending root of V) = somatic afferent.

[1] *Bau der nervösen Centralorgane*, Leipzig, 1885, p. 118.

On the same plate, Fig. 2, I have made an attempt to express diagrammatically the formation of the two great groups of cranial nerves and to indicate the arrangement of the different segments. For this purpose I have not attempted to give an accurate representation of any particular brain, but only to indicate by means of different colours the main peculiarities of the segmental cranial nerves. The colours have the same meaning as given above, and in addition I have marked out the degenerated ganglia of the 1st group of cranial nerves with black lines. The outline of the brain itself and the arrangement of its cavity is drawn from a longitudinal section of the brain of a young dog-fish and is represented somewhat diagrammatically for the purpose of illustrating Part II of this paper. Thus the cerebellum and optic lobes are not intended to be accurate in size but are put in simply for the purpose of indicating their position. For the purpose of comparison with the cranial nerves I have drawn and coloured two typical segmental spinal nerves from the thoracic region as the continuation of the same diagram.

I conclude then that the cranial nerves, with the exception of the optic, olfactory and auditory, are formed from a series of segmental nerves of the same type as in the spinal region, but have been modified so as to form two distinct great groups, of which Group I consists of at least four complete segmental nerves each of which has lost certain components owing to the loss of the parts which they originally supplied; these nerves are—1st segment, oculomotor; 2nd segment, trochlear; 3rd segment, motor part of trigeminal with abducens; 4th segment, facial.

Group II. consists of at least five complete segmental nerves which are both cranial and spinal in origin. The characteristic of this group is not the loss of any particular component but the splitting up of the different components to form the separate nerves known as the vagus, glossopharyngeal, hypoglossal, spinal accessory nerves, together with the sensory part of the trigeminal; and a further characteristic of this group is the remarkable and extensive peripheral distribution of its nerves, a distribution which is partly at all events for the purpose of replacing the lost elements of Group I.

In order then to understand the present arrangement of the vertebrate nervous system some explanation must be found for the formation of these two characteristic groups. I propose then in the second part of this paper to put forward what seems to me a probable explanation of the peculiarities of Group I. of the cranial nerves.

PART II.

A THEORY OF THE ORIGIN OF THE CENTRAL NERVOUS SYSTEM OF VERTEBRATES, TO EXPLAIN THE DEGENERATION OBSERVED IN THE FOREMOST GROUP OF CRANIAL NERVES.

The central nervous system of the vertebrate considered anatomically and morphologically suggests two modes of origin which are apparently antagonistic to each other. On the one hand the segmental arrangement of the nerves, the evidence that not only the nerves but also the groups of nerve-cells from which these nerves arise are arranged not in a continuous chain but metamerically, point directly to the conclusion that the nervous tissue of the animal from which the vertebrates took their origin was arranged in a distinctly segmental manner. On the other hand, the embryological evidence of the formation of the central nervous system, as the folding over of the medullary plate so as to form a simple tube, gives no evidence in favour of any origin from a primitive segmentally arranged nervous system. Any theory then of the origin of the nervous system must take into account not only its segmental arrangement but also its tubular formation.

In all the more recent text-books of human anatomy and physiology great stress is laid on the anatomical and physiological metamerism of the spinal cord, and as is seen in Schwalbe, p. 351, evidence exists to show that the ganglion cell groups of the cord are arranged metamerically.

He quotes Schiefferdecker, who has shown that longitudinal sections in the lumbar region of the dog show that the ganglion cells of the median and lateral groups of the anterior horn are arranged after the fashion of a rosary in such manner that the broad parts, where the ganglion cells are thickest, correspond to the places of exit of the corresponding anterior roots.

He points out also that Freud has noticed that the posterior cells in the lamprey (Hinterzellen), which are supposed by him to give origin to sensory fibres (see p. 157), are arranged in distinct groups separated by considerable distances from each other; and that Stieda noticed the number of nerve cells was very variable in sections of the spinal cord of the eel, many sections being free from nerve cells. Schwalbe concludes that the evidence points to the origin of the spinal cord from

a bilateral chain of ganglia connected together by means of transverse and longitudinal commissures.

This conclusion is strongly confirmed by the evidence given by the origin of the cranial nerves. For in the case of the segmental cranial nerves, which I have already shown are built up on the same plan as the spinal nerves, it is clear that the different groups of cells from which they arise are arranged segmentally. Nothing can be more evident than the groups of cells which give rise to the somatic motor nerves of the IIIrd, VIth and XIIth nerves, in fact the very expressions used to describe these groups as nuclei of the different cranial nerves shows how clearly the discontinuous character of the cell groups of this the most primitive part of the nervous system has been recognised.

The clearest case of segmentation in the cord which I have come across is found in the Sauropsida, for both in crocodiles and birds at the very surface of the lateral region of the cord a group of nerve cells exists which is as strictly metameric as the ganglion cells on the posterior roots of the spinal nerves. I have described this lateral group of cells in the cord of the crocodile in a former paper[1]; and Fig. 5, Pl. XIX illustrates their situation in the cord of the chick.

As far then as that part of the central nervous system is concerned which is connected with the spinal and cranial segmental nerves, we may I think without hesitation consider it as formed by a bilateral chain of ganglia which give origin to a series of segmental nerves and are bound together by connecting fibres both transversely and longitudinally. This forms one uniform system both anatomically and physiologically: a system the members of which, to use Hughlings Jackson's phrase, we may speak of as being on the same level; in addition this system is connected by another set of fibres—fibres spoken of by Hughlings Jackson as extrinsic to this level—to a nervous system of a different character, of higher function, that connection being recognised by the name of the pyramidal tracts, direct cerebellar tracts &c. This nervous system of higher function is not represented in the spinal cord and does not give origin to any outgoing nerves except such nerves of special sense as the optic and olfactory nerves.

If then we fix our attention exclusively upon the nervous elements of the central nervous system, we can describe it as a system composed of a bilateral chain of ganglia connected together by means of longitudinal and transverse commissures,

[1] *Proc. Physiol. Soc.* Dec. 12, 1885. This *Journ.* Vol. VII.

which gives origin to a series of segmental nerves and is connected by means of well-defined commissural tracts with another nervous system of higher function which gives origin to no outgoing nerves except such nerves of special sense as the optic and olfactory nerves.

In addition however to the nervous elements the spinal cord and its extension cranial-wards contains an elaborate system of non-nervous structures, viz. the supporting structures of the cord, and it is here that the significance of the embryological formation of the central nervous system comes prominently forward. If we suppose all the nerve cells, nerve fibres and blood vessels of the spinal cord to be removed we should still have left a tube composed of the supporting structures of the spinal cord and lined by the epithelium of the central canal. In the adult this tube would form a meshwork with nuclei imbedded here and there, the meshes and nuclei becoming closer in the regions of the *substantia gelatinosa centralis* and *Rolandi*, until at the central canal the network becomes compact and forms the epithelial lining of that canal. The formation of this meshwork has been well described by His[1], who points out that it is formed by the modification of layers of epithelial cells of the same kind as those lining the central canal. The protoplasm surrounding the nucleus of the original cell thins out and elongates, forming long threads connecting together the nuclei which are now nearly denuded of their surrounding protoplasm. In Fig. 1, Pl. XIX I reproduce His' figure, which shows clearly the formation of this meshwork, to which he gives the name of myelospongium.

I also (Fig. 2, Pl. XIX) give a drawing of a portion of the substance lining the central canal in the neighbourhood of the infundibulum of a puppy, in which the limbs were fully formed and the groups of nerve cells in the cranial and spinal regions were well defined. It shows clearly the formation of the *substantia gelatinosa centralis* from the original layers of epithelial cells.

Again, Corning[2] has pointed out how the peculiarities of the *substantia gelatinosa Rolandi* are due to the persistence in this position of a concentrated and comparatively unaltered portion of the original embryonic cell formation.

Clearly then the folding over of the medullary plate gives origin not merely to nervous material but also to a tube of

[1] *Op. cit.*

[2] *Archiv f. mikroscop. Anat.* Vol. XXXI.

supporting tissue which was originally formed of compact layers of epithelial cells arranged symmetrically around the central canal.

In the course of development this compact tube becomes broken up into a loose meshwork by the invasion, increase and growth of the nervous elements.

What is the connection between these two structures? If I understand rightly the view which has been held by many embryologists, it is that all the cells of the embryonic tube formed by the folding over of the medullary plate are capable of being converted into nervous tissue, that therefore in the non-nervous parts of this tube we have structures which are potentially nervous, the cells remaining in the embryonic condition although of exactly the same nature as those which have become nervous material.

The view which seems to me the correct one is that in the embryological development of the central nervous system we are observing the simultaneous development of two different organs, the one the nervous system, and the other the tube of supporting tissue the formation of which is not necessarily involved with that of the nervous system. In certain parts of the central nervous system the sole structure formed by the folding over of the medullary plate is the supporting tube which is not nervous and never was nervous, while in other parts the simultaneous formation of nervous material with that of the supporting tube has so complicated the problem that it is difficult to decide which of the embryonic cells form supporting structure and which nervous material.

The evidence both ontogenetical and phylogenetical appears to me to lead to the conclusion that the central nervous system of the higher vertebrates has been formed by the spreading and increase of nervous material over the walls of an original non-nervous tube the cellular elements of which tube, whatever may have been its original function, have been utilised as supporting structures for the nervous elements in those parts where the latter have invaded its walls; while in other parts where no such invasion has taken place the walls of the tube have retained their primitive simple cellular structure or have undergone gelatinous degeneration.

To take first the phylogenetic evidence; the striking characteristic of the evolution of the vertebrate central nervous system is the increase in amount and complexity of that part of the system which forms the brain, so that in the evolution of this part of the nervous system especially we may expect to find evidence which will enable us to decide

whether that increase of nervous material has taken place by the continual growth of nervous matter over an original non-nervous substratum, or whether all parts of it were originally composed of nervous material which has thinned down in places and become non-nervous.

The comparison of the brain of the lowest vertebrate, such as Petromyzon especially in its Ammocœte form, with that of a mammal shows how striking in these two cases is the difference in the extent of the nervous masses situated on the dorsal side of the brain tube. In the former the whole of the dorsal wall is composed of a non-nervous membranous bag which if expanded would be of considerable size, but which by being thrown into a complicated system of folds forms the series of choroidal plexuses described by Ahlborn[1] and other writers and gives to this brain so characteristic and puzzling an appearance. This non-nervous roof to the brain of the lamprey is interrupted by nervous matter in two places corresponding to the two places where the two original constrictions in the brain tube took place by which the three cerebral vesicles are formed, viz. a small band of nervous matter which according to Ahlborn is the commencement of cerebellum and optic lobes and another in the region of the posterior commissure forming the ganglia habenulæ and optic thalami.

On the other hand in the mammal we have the large masses of the cerebellum, corpora quadrigemina, and optic thalami roofing over the brain tube, so that nothing is left of the original membranous roof except the thin roof of the 4th ventricle, the remains of the non-nervous choroid plexuses being enclosed in the 4th, 3rd and lateral ventricles.

In the Elasmobranch, as for instance the dog-fish, we find again that parts of the walls of the brain tube are conspicuously membranous in character; thus we find in the infundibular region a membranous sac known as the *saccus vasculosus*, the membranous roof of the 4th ventricle is more extensive than in the mammal, the choroid plexuses are very conspicuous as is shown in the diagram Fig. 2, Pl. XVIII., and what is especially instructive, we find here on each side of the central cerebellar mass or worm a lateral membranous bag continuous with the roof of the 4th ventricle, the ventral surface of which forms the lining membrane of the two masses of nervous tissue often called the *corpora restiformia* but which G. Fritsch[2] calls the *fimbriæ* and recognises as the commencing formation of the cerebellar hemispheres.

[1] *Zeit. f. wissens. Zool.* Vol. xxxix. p. 191.
[2] *Bau des Fischgehirns*, Berlin, 1878.

In Fig. 8, Pl. XIX I give a dorsal view of the medulla oblongata and cerebellum of a large dog-fish to show the thin membranous roof of the 4th ventricle and its continuation into the two membranous lateral bags which overlie the fimbriæ. In Fig. 7, Pl. XIX I give the appearance of a transverse section through one of the fimbriæ with its overlying membranous roof, to show how the epithelial lining of that roof does not follow the course of the pia mater, but passes into, blends with, and forms the lining of the nervous matter of the fimbria itself; as is also seen, proliferation of these epithelial cells appears to take place where the membranous bag comes into close relation with the nervous substance.

I will not go further into a detailed description here of the manner in which in the lowest vertebrates this membranous non-nervous tube has been folded and compressed in the cranial region by the growth of the nervous material within and around its walls, but will leave that to be dealt with in a subsequent paper upon the central nervous system of the Ammocœte, in which I intend to show how the study of the lowest vertebrate nervous system confirms and strengthens the conclusions to which the study of the highest vertebrate nervous system has led me.

I can see no evidence to show that the thin membranous portions of the roof of the brain are the thinned down remnants of some pre-existing nervous material, and I cannot conceive that a non-nervous epithelial bag should be formed in the lower vertebrates only for the purpose of being converted into nervous material as the animal rose in the scale of evolution. The reasonable explanation therefore appears to me to be what I have already suggested, viz. that the nervous material of the vertebrate central nervous system is situated in definite places outside but in close contact with the walls of a pre-existing non-nervous tube, and that the elements of this non-nervous tube, which is formed by the folding over of the medullary plate, become utilised as the supporting tissue or myelo-spongium wherever the nervous matter comes in contact with it.

Again the evidence of embryology appears to me to point to the same conclusion, for we find that in all classes of vertebrates the nervous matter, both nerve cells and nerve fibres, makes its appearance first on the ventral side of the original tube, so that, as is seen in Fig. 2, reproduced from Kölliker, in the cervical region of the spinal cord of a rabbit 14 days old the dorsal portion of the walls of the original central canal still retains its original character and has not

been invaded by nervous material; the same thing is seen in Fig. 3, representing a section through the 4th ventricle of the embryo of the sheep. In the first case, by the invasion of nervous tissue, the dorsal portion of the original canal is subsequently converted into the

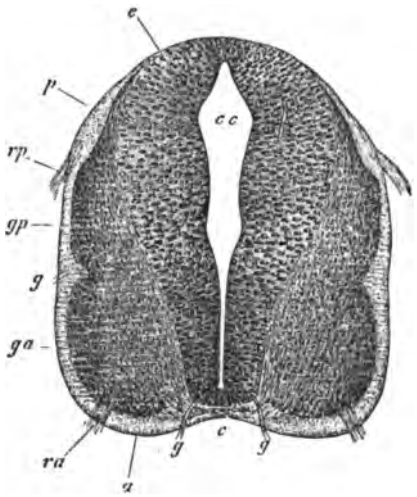

FIG. 2. Section of spinal cord from cervical region of embryo of rabbit (14 days old). (From Kölliker).

In Kölliker's description the letters in the figures have the following meanings. *c.c.* central canal, *a.* and *p.* anterior and posterior columns respectively, *ra.* and *rp.* anterior and posterior roots, *ga.* and *gp.* motor and sensory grey nuclei respectively, *c.* anterior commissure, *e.* apparent epithelium of the central canal.

posterior fissure, the walls of the canal forming especially the substantia gelatinosa centralis and Rolandi; in the second case they remain

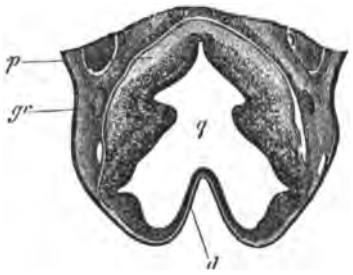

FIG. 3. Section of the brain of a sheep embryo (15 mm. long) through the 4th ventricle. (From Kölliker). *d.* thin roof of 4th ventricle, *q.* 4th ventricle, *p.* white fibres of pyramid (?), *gr.* ganglion-cell masses on the floor of the 4th ventricle.

as modified epithelial structures to form the membranous roof of the 4th ventricle.

The same method of formation, with the same structure, is found in the membranous portions of the brain already spoken of, such as the infundibular sacs of the skate and the membranous enclosures of the fimbriæ of the dog-fish, and the comparison of Fig. 3, Pl. XIX, which represents a section through a part of the *saccus vasculosus* of the skate, with Fig. 1 on the same Plate, shows that the membranous structure formed is in all cases the same in character as the myelo-spongium described by His, which was, as we have seen, formed from the walls of the same embryonic tube. Especially instructive is the evidence given by the formation of the rhomboidal sinus in birds.

At first the embryonic tube is formed in the same manner in this part of the nervous system as in other places, so that Kölliker's picture of the cord of the rabbit, Fig. 4, Pl. XIX, may be used to illustrate the original relation between the nervous and non-nervous parts of the tube here as elsewhere. At a later stage we see as represented in Fig. 5, Pl. XIX, that the white matter of the cord remains separated in the dorsal region by the original layers of embryonic cells; the result being that in the course of growth this mass of embryonic tissue which is not invaded by nervous elements forms a wedge-shaped mass in between the nervous elements on each side.

This tissue is at first composed of the same kind of epithelial cells as those forming the lining of the central canal, each cell possessing a well-defined nucleus in the midst of its protoplasmic contents so that at a period (about eight days) when the nerve cell groups of the anterior horn, the white matter of the cord and the central canal are all well formed, the appearance of a section through this region is as given in Fig. 5, Pl. XIX. Later on a peculiar degeneration takes place in these cells, which consists in the formation of a large quantity of gelatinous material which apparently, just as the fat globules in a fat cell, push the nucleus and the remainder of the protoplasm of the cell to one side, so as to give the appearance of a nucleated mesh-work, the interspaces of which are filled up with gelatinous material. This is represented in Fig. 6, Pl. XIX. At the same time in consequence of the large amount of gelatinous material, this wedge-shaped altered portion of the dorsal wall of the original tube presents the appearance in the adult bird of a jelly-like mass lying upon the spinal cord in this region.

Here it seems to me we see the formation of a modified form of the myelo-spongium of His, which on the one hand has not formed a supporting structure for nervous tissues because it has not been invaded by

nervous elements, and on the other hand for some reason or other has not thinned down like the corresponding structures in the cranial region, but on the contrary has increased in thickness and undergone gelatinous degeneration.

The difference between this part of the nervous system as seen in birds and that seen in other vertebrates is clearly not due to atrophy in the case of the former of any special nerve tissues which are developed in the latter, for birds are supplied in the lumbar just as much as in other spinal regions with the same nerve structures, possessing the same functions, as other warm-blooded animals. There is no evidence here of any loss of nervous mechanism, but only a difference of arrangement of the nervous material in consequence of which the dorsal wall of the original tube has been left free from the admixture of nervous matter, and having lost its original function has undergone gelatinous degeneration.

The whole evidence then, embryological as well as phylogenetical, appears to me to point to the conclusion that the spinal cord is formed of a non-nervous tube, the walls of which have become invaded with nervous material.

Again, the embryological evidence of the order in which this nervous material makes its appearance is very striking and very suggestive.

According to the account given by Balfour, the white matter appears first in Elasmobranchs as a layer on the ventral and lateral parts of the spinal cord, but does not extend to the dorsal surface; at the same time the anterior white commissure is formed. About the same time two large bilateral ventral masses of nerve cells appear which are formed on the outer anterior part of the original thick walled tube as seen in Fig. 4, Pl. XIX, so that at this time, before the dorsal coalescence of the walls of the canal to form the posterior fissure, the nervous elements are represented by a series of symmetrically situated bilateral ganglion masses connected together by longitudinal and transverse commissures, the whole being situated within the walls of, but ventrally to, the non-nervous tube already spoken of. With the formation of the posterior fissure the nervous material spreads round to the dorsal side of the tube, and finally the chief evidence of the original tube is to be found in the peculiar structure of the *substantia gelatinosa centralis* and *Rolandi*.

The difficulty of deciding which of the elements of the original embryonic tube will form nervous material and which will form sup-

porting structure consists in the absence of any histological means of discriminating between the two structures at an early time of development: much has been written about the first development of nerve cells and nerve fibres in the spinal cord, but I doubt very much whether we can go much further than the observations of His[1], viz. (1) that all the cells of the embryonic tube do not form nervous material; (2) that all the motor nerve fibres arise as prolongations of the motor nerve cells; and (3) that the motor nerve cells as soon as they can be recognised are always situated in a perfectly definite place in the embryonic tube, viz. in the outer part of the tube (Mantelschicht) and not in the inner part (Innenschicht).

Up to this point I would submit the following possible explanation of the ancestral history of the spinal cord.

It was originally composed of a bilateral chain of ganglia, situated ventrally to a non-nervous tube, the parts of each chain being connected together by commissures also situated ventrally to this tube. By the increase and spreading round of the nerve cells and nerve fibres to the dorsal side the original tube was so invaded with nervous elements as to have lost its original character and become the supporting structure of the spinal cord; leaving as the most marked indication of its original character the epithelial lining of the central canal and the peculiar structure of the *substantia gelatinosa centralis.*

Tailwards this tube emerges free from its encasing mass of nervous matter as the neurenteric canal and its walls are continuous with those of the alimentary canal. Headwards this tube passes into the 3rd ventricle and has apparently no anterior opening.

In the cranial region the formation of the original tube by the folding over of the medullary plate is of the same character as in the spinal region, the main difference being the larger size of the cavity formed. The cavity, as in the spinal region, is throughout lined with the epithelium of the central canal and the *substantia gelatinosa centralis* is formed in the same way as in the spinal region. We see then in the cranial region that the tube of supporting tissue exists of the same character and formed in the same way as in the spinal region.

What then about the nervous elements, are they the same here as in the region of the spinal cord? To my mind clearly not. In this part

[1] *Anat. Anzeig.* 1888, p. 499.

of the nervous system we find, as already pointed out, both physiologically and structurally, two different nerve systems bound together. On the one hand the continuation of the metameric groups of nerve cells with their connecting fibres and their outgoing segmental nerves, which we have found to constitute the essential part of the spinal cord, forming a nerve system of the same level as that of the cord, a nerve system which we may call the cranial portion of the spinal cord nervous system; on the other hand a nerve system of different structure and higher function, which is connected by well-defined nerve tracts with the nervous system of the spinal cord; this nervous system of higher function, which we may term the brain proper, is not found at all in the spinal cord but only the tracts such as the direct cerebellar, the pyramidal &c., which connect the spinal cord nervous system with this brain.

How are these two different systems situated in the cranial region? We see that both embryologically, anatomically and physiologically the spinal cord nervous system in the cranial region arises in the same way as in the spinal region and is directly continuous with the nerve tissue of the spinal region; the metameric ganglion masses with their investment of nerve fibres arise here in pairs, connected by a ventral commissure on the ventral side of the original tube just as in the spinal cord. The nervous matter spreads laterally just as in the spinal cord, but the great distinction between the two places is found in this, viz. that the elements of this nervous system do not as in the spinal cord invade the dorsal side of the tube; they remain on the ventral and lateral sides of the tube forming the floor of the medulla oblongata and of the hind- and mid-brains. Further, the anterior limit of this system is very clearly defined; the nucleus of the IIIrd nerve is the limit of the groups of nerve cells connected with segmental nerves built up on the spinal nerve type; the tuber cinereum and infundibulum mark clearly the limit of spinal cord structure in the cranial region. In the diagram Fig. 2, Pl. XVIII I have represented the manner in which the spinal cord structure becomes limited in the cranial region to the ventral side of the central canal. If then we speak of the continuation of the spinal cord structure up to the infundibulum as the floor of the original tube in the cranial region, we have left on the dorsal side forming the roof of the tube special nervous structures forming the brain proper.

These masses of nervous structures formed in the roof form a separate chain of ganglion masses separated from each other by the thin membranous, almost non-nervous, walls of the original tube. Thus

we see firstly the membranous roof of the IVth ventricle passing into the *velum posterius,* then the ganglionic mass of the cerebellum connected with the rest of the nervous system by means of the inferior middle and superior peduncles; then the semi-membranous *velum anterius* or valve of Vieussens, upon which, as a sign of the striking nature of the change which has occurred in the past history of the vertebrate, the IVth nerve has become fixed; then the ganglionic masses of the optic lobes, then again a thinned-out portion where in the past history of the vertebrate the invertebrate eye now known as the pineal gland took its rise; close to this, situated laterally, the two conspicuous most primitive ganglionic masses known as the optic thalami, and then anterior of all the cerebral hemispheres. In fact we find this striking fact, that the nervous matter formed in the roof of the original tube in the cranial region is composed of 4 separate paired masses of nervous tissue of different structure and higher function than the nervous system formed on the floor of the tube; the different ganglion masses are connected with each other and with the spinal cord nervous system by a perfectly well recognised system of commissures, already spoken of as the nerve tracts extrinsic to the level of the spinal cord. Further, it is to be noticed that the increase in the size of these nerve tracts coincidently with the great increase and development of these groups of dorsal ganglion masses constitutes the most striking feature of the development of the vertebrate nervous system.

We see then that it is possible to look upon the central nervous system as being built up around and within the walls of a tube somewhat as follows. The nervous system from which the vertebrate system was formed was originally composed of a bilateral chain of ganglia situated on the ventral side of this tube, the nervous material of which has subsequently grown round and enveloped the tube both dorsally and ventrally along its whole length with the exception of its anterior end; here the ventral chain of ganglia instead of spreading round to the dorsal side of the tube is connected by means of strong encircling commissures, forming a commissural collar around the tube, with a series of ganglia lying on the dorsal side of the tube whose function is of a higher character than that of the ventral chain and which give rise to no outgoing nerves except those of such special senses as sight and smell.

Clearly this description is the description of the typical form of nervous system found among invertebrates in which the dorsal ganglion

masses of the cranial region with their connections with the optic and olfactory nerves are the supra-œsophageal ganglia; the nervous matter which gives origin to the segmental cranial nerves and to the spinal cord forms the infra-œsophageal ganglia and ventral chain; and the crura cerebri, peduncles of the cerebellum, and the other tracts which are extrinsic to the level of the ventral ganglionic chain form the œsophageal collar. The necessary conclusion to this comparison is that the tube of supporting tissue around and within which the nervous system is formed, with its extraordinary continuation as the neurenteric canal into the present alimentary canal, was originally the whole or part of the alimentary canal of the invertebrate from which the vertebrate ancestor arose; and it still further follows that this tube must have had an anterior opening as well as a posterior one, the œsophagus must have ended in a mouth just as the intestine in an anus. Examination of the region in which the mouth was most likely to have been situated, viz. the infundibular region, where the notochord and the nervous tissue which corresponds to the infra-œsophageal ganglia terminate, has led me to the discovery of what I believe to be the remains of the terminal œsophageal tube. My original discovery was made on the brains of adult sheep which were taken out of the animal immediately after death, and the portion of the base of the brain which contained the infundibulum with the pituitary body attached and the tuber cinereum was cut out and placed in 1 % osmic acid; the pituitary body being turned up towards the optic chiasma and the piece of tissue hardened in that position. In this way the region of the tuber cinereum was well exposed to view and it was easy to cut a whole series of sections transverse to the long axis of the brain, beginning at the cavity of the infundibulum and passing onwards to the corpus mamillare. These sections showed the existence of a canal leading from the cavity of the infundibulum towards the corpus mamillare; a canal which lies quite on the surface of the brain and occupies the greater portion of the length of the tuber cinereum. This canal is lined with epithelium continuous with that of the IIIrd ventricle and of the infundibulum; its walls are composed of substance similar to the substantia centralis gelatinosa; the further away from the infundibulum the more is its cavity closed by the approximation of its walls, and the nearer it comes to the surface, until at last, when completely closed, it vanishes at the very surface where a number of blood vessels from the pia mater pass in to the brain substance. Its appearance is exactly that of an open tube which has been bent down on the surface of the brain and so its open extremity been obliterated by the coming

together of its walls; the appearance of a tube lying flat is much enhanced by the presence of a row of blood vessels between it and the brain proper. In Fig. 3, Pl. XX, I represent a series of sections through this canal at different distances from the infundibulum.

The examination of other cases is very instructive. Thus in the skate the series of sections through this region shows how the terminal portion of this tube has been fixed down and partially obliterated while the next portion has been distended and folded to form the saccus vasculosus. I have also found the same appearance in the brain of the dog-fish; the diagram on Pl. XVIII is drawn from one of the series of longitudinal sections through the brain of a young dog-fish and is a fairly accurate representation of the shape of the brain cavity; the dorsal masses of nervous matter are not drawn to scale but simply indicated so as to show roughly the relation of parts. In that figure the position of this folded down tube is clearly shown.

In Fig. 1, Pl. XX, I give a sketch of the ventral surface of the brain of a dog-fish to show the position of this folded down tube and the position in which the brain was hardened for the purpose of making a series of sections through this tube. The pituitary body, the saccus vasculosus and the lobi infundibuli were all turned back on to the optic chiasma as far as possible; the brain was then placed in picric acid, hardened, stained, and a series of sections cut through the part indicated by the lines drawn across Fig. 1.

Fig. 2, Pl. XX, represents a selection out of the whole series of transverse sections through this part of the brain. They disclose clearly the fact that the cavity of the infundibulum not only forms the cavity of the lobi infundibuli and of the saccus vasculosus but is continued on the surface of the brain backwards beyond the limits of the saccus vasculosus as a tube which becomes more and more superficial. An examination of the separate sections shows that this tube forms a distinct projection on the surface of the brain and at first it possesses a conspicuous lumen lined with epithelial cells of the same character as those lining the rest of the central canal. In the 3rd section represented the last remains of the cells lining the lumen is seen, but as is seen in the 4th, 5th and 6th sections the walls of the tube not only continue to appear in each section but come to lie more and more outside the nervous matter, so that in the last two sections the continuation of the walls of this tube is seen as a small mass of peculiarly structureless tissue entirely surrounded by pia mater and blood vessels and so lying freely on the surface of the

contiguous nervous matter. Examined with a high power this tissue presents the appearance of a remarkably homogeneous gelatinous-looking material free from nuclei in which a meshwork of fine lines is seen.

Again, in the young lamprey this folded down tube is most conspicuous and beautifully shown in the series of sections which I have made. As however I propose to deal in a separate paper with the evidence which the investigation of the lamprey has given in confirmation of the views expressed in this paper, I will only mention the fact of the existence of this tube here, in order to point out that this same structure is found in both the highest and the lowest vertebrate. My suggestion then is that the terminal part of the œsophagus has been obliterated by being folded down on the infra-œsophageal ganglia while the next portion of the œsophagus has been dilated to form the infundibulum with the glands of the pituitary body lying on the anterior lip of the original mouth and œsophagus. In order to illustrate my meaning, I here reproduce, Fig. 4, Pl. XX, Dohrn's picture[1] of the nervous system of a young Limulus in which, as is seen, the mouth and œsophagus are lying on the chain of infra-œsophageal ganglia in exactly the same way as this tube in the vertebrates of which I have been speaking.

If this tube be really, as I have suggested, the remains of the original mouth and œsophagus, then I believe that the vertebrate nervous system is brought into complete harmony with that of the invertebrate, and although I know that my view will meet with strenuous opposition from many morphologists who hold very different views upon the origin of the vertebrate nervous system, yet I am emboldened to put it forward not only because it appears to me to follow legitimately from the anatomical and physiological facts which I have brought forward, but also because so many previous investigators have felt the necessity of harmonising the vertebrate and invertebrate nervous systems. Thus Balfour is strongly in favour of looking upon the fore-brain of the vertebrate as the pre-oral ganglion of the invertebrate; Dohrn was so anxious to pierce the brain with an œsophagus that he at one time suggested that the original mouth was situated dorsally and that the old œsophagus passed through the roof of the 4th ventricle and so into the alimentary canal through the infundibulum, thus turning over the ancestor of the vertebrate and making him crawl on his back. It is unnecessary to mention the names of other morphologists

[1] *Jenaische Zeitschr.* Bd. VI. Taf. XV. Fig. 10.

who have made attempts in this direction, for the desire of piercing the brain of the vertebrate with an œsophagus has been expressed again and again in the writings of many distinguished comparative anatomists from Owen downwards.

I recognise fully the many difficulties which require explanation before this view can receive general acceptance, and especially the difficulty of accounting for the present alimentary canal. I beg therefore of my readers to abstain from criticism upon this point until I have published my paper to explain the peculiarities of the hindmost group of cranial nerves; for in that paper I shall endeavour to explain the formation of the present alimentary canal just as in this paper I have endeavoured to account for the old invertebrate alimentary canal. So also I have not touched upon the embryological difficulties which apparently stand in the way of the acceptance of this view of mine; for I consider that their discussion ought to come, and will come best, after some conclusion upon the ancestor of the vertebrate has been arrived at upon anatomical, physiological and phylogenetic grounds, and by that time, if we may judge from the change of opinion which is now going on, the embryological standpoint will have changed so much that perchance many of the present difficulties will be found to have vanished.

I ought perhaps to mention here that in a paper published during this last summer Bland Sutton[1] has suggested that pathological cystic malformations of the spinal cord point to the cord as being a modified gut. The view which I have put forward in this paper was in no way suggested by this paper of Bland Sutton's, for I had already worked out and written out a manuscript embodying my views during the Christmas Vacation of 1887; and indeed the opinions which I held were well known to my friends and many students at Cambridge during the summer and autumn of 1887. On the advice however of friends I determined not to publish the whole manuscript at that time but only the part concerning the structure of the cranial nerves which appeared in the *Proceedings of the Royal Society.*

Sutton's paper is to me most valuable because it confirms, from an entirely different method of investigation, the importance of looking upon the non-nervous elements of the spinal cord as possessing a separate individuality from the nervous elements.

Finally, we see that a self-evident and straightforward reason for the degeneration of certain components of the foremost groups of cranial

[1] "Brain," Vol. x. p. 429.

nerves, *i.e.* nerves which arise from the original infra-œsophageal ganglia, is afforded by the loss of function of the invertebrate alimentary canal and the mouth parts in connection with it. It is to my mind very suggestive that a part at all events of the degenerated material in the IIIrd nerve is apparently in direct connection with the large group of cells known as the *substantia nigra*, for the direction of the processes of many of these cells is parallel to the direction of the fibres of the IIIrd nerve, and the cells themselves are imbedded in the continuation of the degenerated material belonging to the IIIrd nerve.

The groups of cells known as the substantia nigra have long been a stumbling block to anatomists; their strongly pigmented character points directly to a cessation of function on their part; they have not been traced into connection with any definite tracts of nerve fibres; and their position on the crura cerebri is a peculiar one and is not homologous with any of the groups of nerve-cells which are in connection with the spinal and cranial segmental nerves. If however we look upon the nervous system from the point of view suggested in these pages, we see that the crura cerebri form the main commissures connecting the supra- and infra-œsophageal ganglia, and that a group of cells situated upon these œsophageal commissures belonging neither to the supra-œsophageal or infra-œsophageal systems would correspond exactly to the stomato-gastric ganglion which in crustaceans forms the main nervous supply of the mouth and alimentary tract. If then it be allowable to speak of the parts of the vertebrate nervous system in invertebrate phraseology, I would suggest that with the alteration of function of the original invertebrate alimentary canal is associated the pigmentation of the cells of the stomato-gastric ganglion which supplied that canal with nerves, and therefore may perhaps still supply the myelo-spongium with nervous elements, and also the degeneration of certain parts of the nerves which arise from the infra-œsophageal ganglia.

I have mentioned the word Crustacean in connection with the nerve supply of the invertebrate alimentary canal, and it appears to me that the theory which I have put forward fits in best with an original alimentary canal of the crustacean type. Thus in Fig. 3A, Pl. XVIII, I give a diagram of the relation between the nervous system and the alimentary canal of the Crustacean with its turned down œsophagus, large cephalic simple stomach and straight simple intestine. In such an animal as *Sphæroma* we find according to Bellonci[1] that the supra-

[1] *Archiv Ital. de Biolog.* Vol I. p. 176.

œsophageal ganglia are separable into 3 segments: 1. a superior segment or cerebrum; 2. a middle segment which gives origin to the optic and olfactory nerves; and 3. an inferior segment which is situated upon the œsophageal commissures and forms the stomato-gastric ganglion and gives origin to the nerves of the external antennæ. Such an arrangement I have represented diagrammatically in Fig. 3 A.

In the vertebrate the folding over of the medullary plates in the cranial region forms first a simple large bag in connection with the straight narrow tube of the spinal cord. This stage then of embryological development corresponds to Fig. 3 A. The next important occurrence embryologically is the formation of the 3 cerebral vesicles. At first the cephalic bag is constricted in one place so as to form two lobes, and later on another constriction appears in the posterior lobe by which 3 instead of 2 vesicles are formed. The first constriction takes place at that spot which afterwards forms the posterior commissure, and here we see the ganglia habenulæ are formed in the lamprey, ganglia which are clearly in connection with the pineal eye. From the ganglia habenulæ across to the ventral side of the brain in the lamprey run two well-marked nervous commissures known by the name of Meynert's bundles.

Upon the view put forward in this paper this first constriction represents simply the formation and growth in this position of nervous matter which is homologous with the middle segment of the supra-œsophageal ganglia of *Sphæroma*; which therefore is in connection with the original organs of sight, i.e. with the pineal eye.

The 2nd constriction, which occurs subsequently to the first, is in the position of the valvula cerebelli or valve of Vieussens, and is therefore associated with the crossing over of the IVth nerve from the ventral to the dorsal side; here at first according to Ahlborn[1] in Ammocœtes a deep inward projecting fold of the membranous roof of the brain is the only indication of the position of cerebellum and optic lobes. If then we imagine in the Ammocœte the 3 choroid plexuses inflated so as to get rid of their folds and creases, Fig. 3 B would represent not only the embryological stage of the formation of the 3 cerebral vesicles but also the corresponding phylogenetic stage as shown by the brain of the Ammocœte.

From this stage to that represented by the Elasmobranch brain and so on to the mammal the steps are easy; owing to the increase of the nervous material and to the limited space in the cranial cavity, the free

[1] *Op. cit.*

portions of the original cephalic stomach are thrown into folds forming the choroid plexuses and the other membranous parts of the brain already spoken of in this paper. Thus, as is seen in Fig. 3 C or Fig. 2, Pl. XVIII, a loop of the bag of the 3rd vesicle forms the internal lining of the worm of the cerebellum. This very formation of the worm of the cerebellum in the Elasmobranch produces of necessity the two lateral membranous folds shown in Fig. 8, Pl. XIX. These in their turn become surrounded with nervous matter and then form the cerebellar hemispheres. In front of the valvula cerebelli the nervous matter grows over the membranous walls of the 2nd vesicle and forms the optic lobes.

By the growth of the cerebral hemispheres and the increase in size of the two lateral masses known as the optic thalami, the membranous portion of the 1st cerebral vesicle is thrown into folds which project into the 3rd ventricle and form the choroidal plexuses of the 3rd and lateral ventricles, as is seen in Fig. 2, and Fig. 3 C, Pl. XVIII. In this way it appears to me perfectly possible to construct the vertebrate nervous system from one of the Crustacean type.

Before however it is possible or advisable to speculate further upon the changes associated with the loss of the old alimentary canal, it is necessary to have some conception of the manner in which the present alimentary canal arose; in other words to attempt to explain the peculiarities of the hindmost group of cranial nerves, for it is evident that their peculiarities are associated with the formation of the new, *i.e.* the present alimentary canal, just as much as those of the foremost group were associated with the loss of the old canal. Such an attempt may well form the subject of a separate paper, which I hope before very long to be able to publish.

CAMBRIDGE,
March 20, 1889.

DESCRIPTION OF FIGURES. PL. XVI—XX.

PLATE XVI.

Fig. 1. Portion of a section through the IIIrd cranial nerve of the dog, to show how the smallest fibres in the nerve congregate together at the periphery, preparatory to passing out into the oculomotor ganglion.

Fig. 2. Portion of a section through the middle of the oculomotor ganglion, to show that there are no large nerve fibres in the ganglion. (From the same series as Fig. 1.)

Fig. 3. Section through some of the short ciliary nerves immediately after their exit from the oculomotor ganglion. (From the same series as Figs. 1 and 2.)

Fig. 4. Section through some of the rootlets of the VIIth cranial nerve of the dog, to show the presence of the large fibres in these rootlets,

Fig. 5. Section through the IVth cervical nerve of the rabbit at the origin of the phrenic nerve, to show the fibres of the phrenic nerve (B) separating out from the larger fibres of the IVth cervical nerve (A).

PLATE XVII.

The sections are all carefully drawn from osmic acid preparations of the nerves of man.

Fig. 1. Transverse section of rootlet of IIIrd cranial nerve to show degenerated ganglion. (Zeiss A, Oc. 4.)

Fig. 2. Three transverse sections of IVth cranial nerve. (Zeiss A, Oc. 2):

a. Section near exit of nerve from valve of Vieussens, showing degenerated tissue arranged so as to form a sheath around the functional medullated nerve-fibres.

b. Section of nerve farther away from point of exit, showing the formation of the degenerated ganglion.

c. Section of nerve peripheral to the ganglion. The degenerated tissue has almost entirely disappeared.

Fig. 3. Transverse section of VIIth cranial nerve to show degenerated ganglion. (Zeiss A, Oc. 2.)

Fig. 4. Longitudinal section of a rootlet of IIIrd cranial nerve to show the structure of the degenerated material. (Zeiss D, Oc. 2.)

Plate XVIII.

Fig. 1. Section of the medulla oblongata (after Edinger). The letters A, B, C, D, E have the same meanings as in the diagram of the spinal cord on p. 155.

Fig. 2. Diagrammatic representation of the arrangement of the segmental cranial nerves. The diagram represents roughly a longitudinal section through the brain of a young dog-fish, upon which the general plan of arrangement of the cranial nerves has been drawn as explained in the text.

Fig. 3. These three diagrams illustrate the possible origin of the vertebrate nervous system as suggested in the text.

3 A. Diagram of the relation between the Crustacean alimentary and nervous systems.

3 B. Diagram of relation between the nervous and non-nervous parts of the central nervous system in the Ammocœte. It illustrates also the formation of the three cerebral vesicles from the simple stage represented in Fig. 3 A.

3 C. Diagram of relation between the nervous and non-nervous parts of the central nervous system in the Elasmobranch and in the higher vertebrates generally. It illustrates the formation of the choroid plexuses and the increasing invasion of the walls of the original cephalic stomach by the increase in the amount and complexity of the nervous material.

Plate XIX.

Fig. 1. The supporting tissue or myelospongium of the spinal cord (copied from His).

Fig. 2. Portion of a section of the lining of the central canal in the region of the infundibulum of a puppy, to show how the original compact layers of epithelial cells spread out to form the substantia gelatinosa centralis.

Fig. 3. Section of the wall of the saccus vasculosus in the adult skate.

Fig. 4. Reproduction of Kölliker's drawing of the spinal cord of the embryo of the rabbit, to show how at this stage the dorsal part of the tube is free from the admixture of nervous material.

Fig. 5. Section of cord in chick (about 8 days) through the rhomboidal sinus to show the position of the groups of motor ganglia, and the manner in which a wedge-shaped mass of the original epithelial embryonic tissue remains on the dorsal side of the central canal free from admixture with nervous material.

Fig. 6. Appearance of this wedge-shaped dorsal mass of embryonic

tissue in the more adult condition. The spaces formed by the nucleated meshwork are filled with a homogeneous gelatinous material.

Fig. 7. Section through the nervous matter forming the fimbria of a dog-fish, with its overlying membranous roof. The epithelial layer of the membranous roof is seen to be continuous with and to form the lining epithelium of the projections of nervous matter, while the pia mater as shown by the thin lines does not follow the course of the epithelial layer.

Fig. 8. Dorsal view of the medulla oblongata and cerebellum of a dog-fish. *cb.* worm of the cerebellum, *l.* the two lateral membranous pouches which form the roof of the commencing cerebellar hemispheres or fimbriæ, *d.* membranous roof of the IVth ventricle which is continuous with the two lateral membranous pouches.

Plate XX.

Fig. 1. Ventral surface of the brain of a young dog-fish; the pituitary body and saccus vasculosus are turned back on to the optic chiasma. The lines drawn across the figure indicate the position of the series of sections from which the drawings in Fig. 2 were made.

Fig. 2. A selection out of the whole series of sections indicated in Fig. 1, to show how the cavity of the saccus vasculosus is continued as a tube on the surface of the brain; the last three sections show how the continuation of this tube at its termination is separated from the brain by pia mater and blood vessels.

Fig. 3. A selection out of a series of sections through the tuber cinereum of the sheep, to show the existence of a tube, the walls of which gradually close, which lies on the surface and passes from the infundibulum towards the corpus mammillare.

Fig. 4. Diagram of the arrangement of the nervous system and of the alimentary canal in the young Limulus. (Copied from Dohrn.)

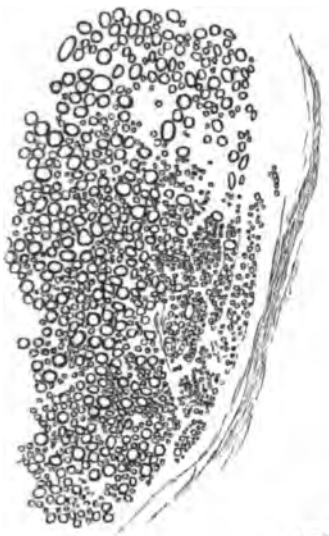

Fig. 1.

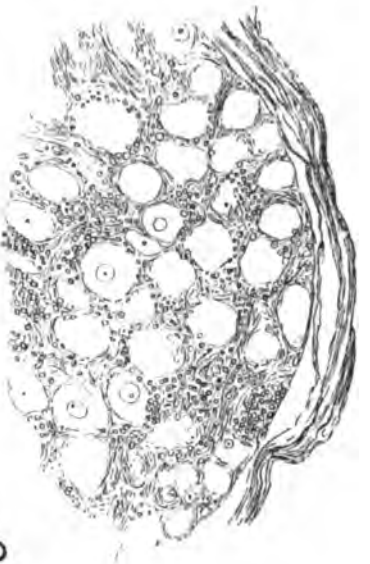

Fig. 2.

Fig. 5.

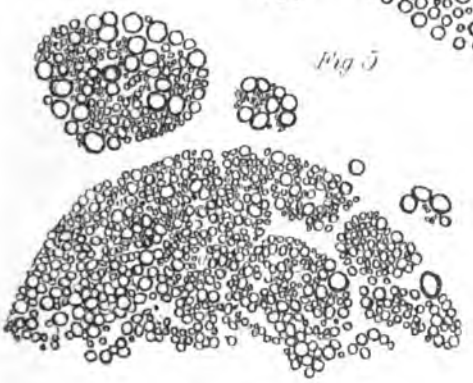

Fig. 4.

Fig. 3.

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Fig.1.

Fig.2.a

Fig 2 b.

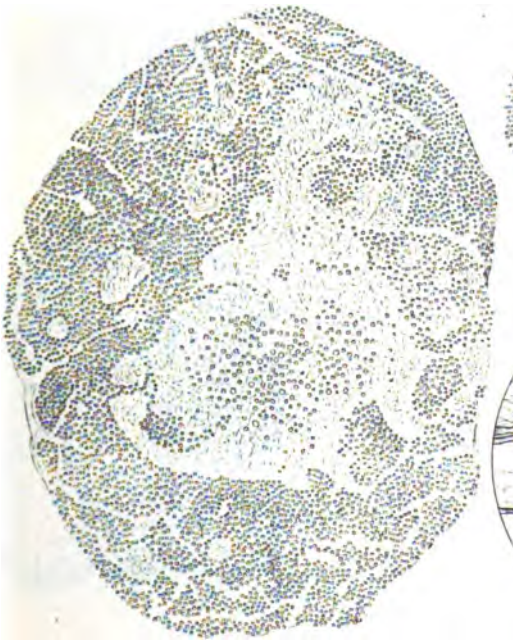

Fig.3.

Fig 2.c.

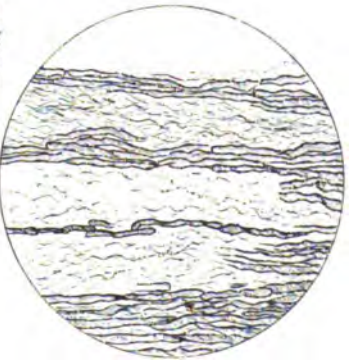

Fig.4.

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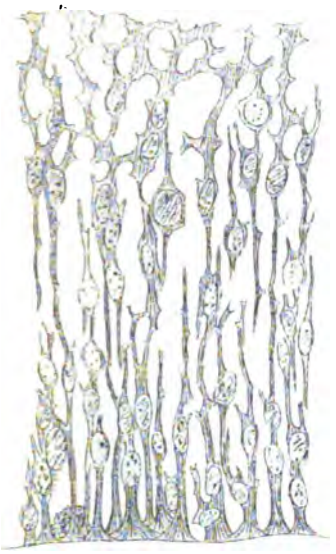

Fig. 1

Fig. 2.

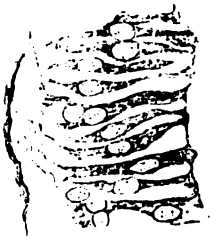

Fig. 3.

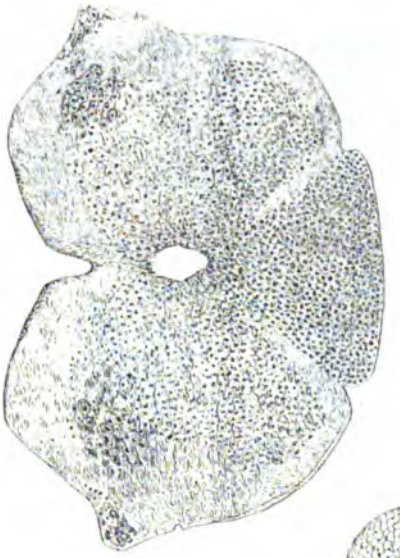

Fig. 5

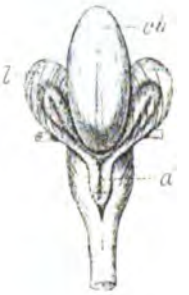

Fig. 8.

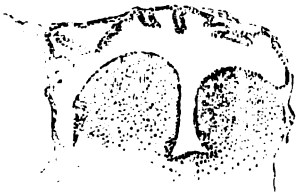

Fig. 7.

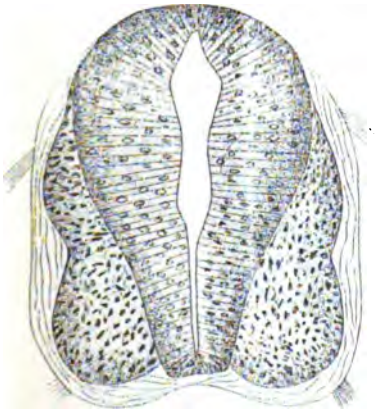

Fig 4.

Fig. 6.

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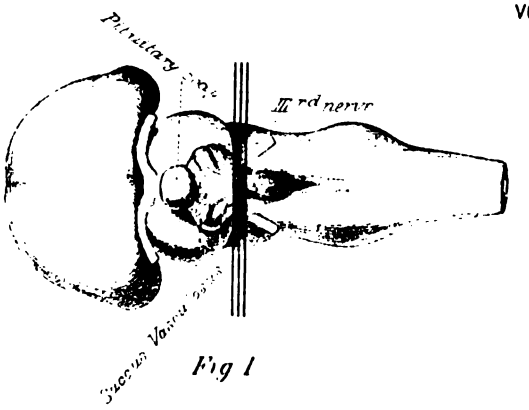

Fig 1

Fig. 2.

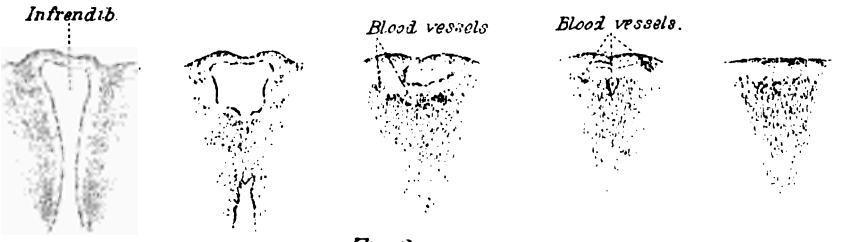

Fig. 3.

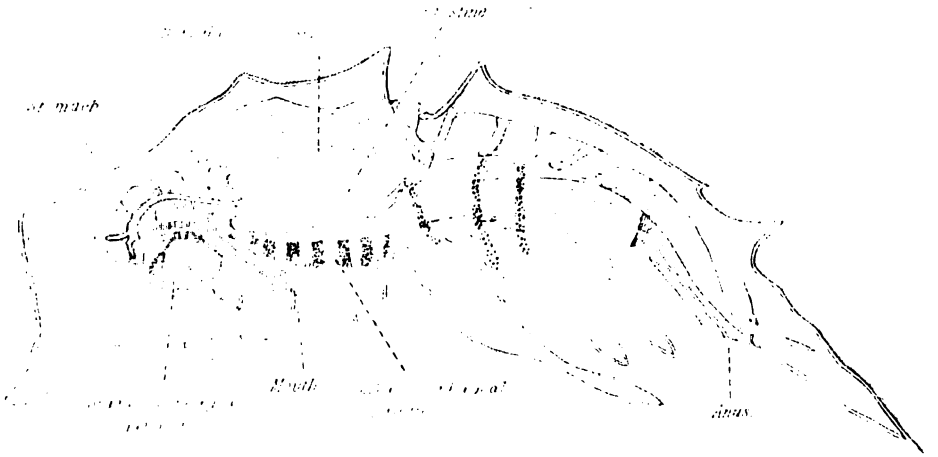

Fig. 4.

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OBSERVATIONS ON HUMAN BILE OBTAINED FROM A CASE OF BILIARY FISTULA By S. MONCKTON COPEMAN, M.A., M.B. Cantab., *Demonstrator of Physiology*, AND W. B. WINSTON. Pl. XXI.

(*From the Physiological Laboratory of St Thomas' Hospital.*)

THE number of cases of biliary fistula in man in which accurate observations have been made, with regard to the somewhat doubtful points of the amount of secretion of the bile, of its chemical constitution, and of the variations, if any, in the rapidity of the flow, are very few; and in nearly all of those which have been recorded malignant disease has been present, or there has been some other complication which renders such observations of comparatively little value from a physiological point of view. This being so, it is possible that the following notes on a case in which apparently no such sources of error were present, and in which the observations extended over a lengthened period, may be worth recording.

The patient, a woman aged 26, was admitted into St Thomas' Hospital in February 1888, suffering apparently from an attack of biliary colic, and came under the care of Dr Bristowe to whose kindness we are indebted for permission to publish our observations on the case.

She had had several somewhat similar attacks during the six months prior to admission, and she had noticed a small hard lump in the region of the gall-bladder, which increased in size, and became very tender and painful after each attack. Both before and after admission, the fæces were carefully examined for gall-stones, but without success.

She was considerably jaundiced, the conjunctivæ being very yellow, and both bile-salts and bile-pigment were present in the urine, while the fæces were of the typical clay colour. After a short stay in the medical ward she was transferred to the care of Mr Sydney Jones, who cut down on the gall-bladder, which was much distended, and after stitching it to the edges of the wound, laid it open, when about six or seven ounces of a glairy semi-transparent fluid, quite free from biliary colouring matter, was evacuated, which under the microscope was found

to contain a number of leucocytes, together with a few crenated red blood corpuscles. No gall-stone could be discovered in the common or cystic ducts.

On the day following the operation, the dressings were found to be stained with a dark-green fluid which was evidently bile, the amount of which increased day by day. At the same time the jaundice disappeared little by little and the urine now gave no bile reaction. The stools remained of a greyish colour, and from that time onwards there was no trace of bile in either urine or fæces.

The wound gradually healed, except at one point from which the bile continued to be discharged, so that it was determined to collect it, and through the courtesy of Dr Bristowe, the opportunity was obtained of examining it before the patient underwent any further surgical interference that might be deemed necessary.

The results noted are based on observations extending over nearly a couple of months, when they were interrupted by the death of the patient, caused by biliary toxæmia from reabsorption of the bile, after plugging the fistulous opening, for hæmorrhage, which unfortunately supervened on an attempt to open up anew a path for the bile into the duodenum, by passing a probe along the common duct.

At the post-mortem examination, the fistulous opening, a vertical slit half an inch in length, which was situated an inch below the costal margin on the right side, was found to communicate with a somewhat contorted, thick-walled passage, along which a probe could be passed for about 3½ inches in a direction upwards and backwards. This canal, which represented the shrunken interior of the gall-bladder, had a uniform calibre of less than half an inch, and was connected above with the hepatic duct and the common bile duct, the condition of which however could not accurately be made out.

A portion of the liver, with the remains of the gall-bladder, the hepatic and common ducts, the pancreas and a few inches of the duodenum, was removed and handed over to Mr Shattock for more careful dissection and preservation as a museum specimen. On examination he found that the liver, except that its substance was stained with bile and that the main bile-ducts were somewhat dilated, was perfectly normal and that there was no stricture or new growth of any kind along the course of either cystic or common duct. Quite at the end of the common duct, just at its junction with the intestine, a calculus was found firmly impacted, which measured 1·6 cm. in its longitudinal diameter, and 1·3 cm. in its longest transverse diameter.

The common duct was dilated particularly at the point of junction with the cystic duct, where, when it was laid open, the internal circumference was found to be 3·3 cm. From the size of the calculus it seemed impossible that anything short of a surgical operation could ever have dislodged it from the position in which it was found.

Amount of Secretion in 24 hours.

As soon as the fistula became established, as described, a glass cannula was accurately fitted into the wound, and by means of an india-rubber tube, the bile was led into a bottle (placed on the floor beside the bed), through the cork of which passed two glass tubes;—one reaching quite to the bottom of the bottle, where it ended in a fine nozzle, being connected up with the cannula, the other a short one to permit the exit of the contained air as the bile flowed into the bottle.

The length of the column of fluid appeared to have the effect of preventing the ingress of micro-organisms, as the wound, which was dressed over the cannula with salicylic wool and iodoform, remained quite aseptic, and the patient's temperature, after a few days from the date of the first operation, until just before her death, never rose appreciably above the normal.

As there was no loss into the dressings, and no trace of bile in either urine or fæces, it is evident that the whole amount of the secretion of the liver was collected, and for more than a month the total quantity secreted during the previous 24 hours was carefully measured at 10 p.m.

The measurements were as follows, taking the first and third weeks of June as examples:—

June 1	.....	26¼ ozs.
„ 2	.....	28½ „
„ 3	.....	27½ „
„ 4	.....	31 „
„ 5	.....	34 „
„ 6	.....	29 „
„ 7	.....	28½ „

On June 4 the patient commenced taking pills of purified ox-gall, in doses of ten grains thrice daily, and at first the amount of the flow appeared to be somewhat increased by the treatment, but, as shown, the amount again fell, after three days, to about the same as before.

The pills were discontinued after a fortnight, without causing thereby any noticeable lessening of the flow, as is shown by the record of that week :—

June	14	27 ozs.	
„	15	24 „	
„	16	28 „	
„	17	24 „	
„	18	26 „	pills taken off.
„	19	27 „	
„	20	26 „	

The amount afterwards ranged between 24 ozs. and 28½ ozs., the average being about 27 ozs. or 779·625 c.c., an amount very considerably in excess of that recorded in somewhat similar cases by Ranke (652 c.c.), v. Wittich (532·8 c.c.) and Yeo and Herroun (374·5 c.c.) especially when the weight of the patient is taken into consideration, as on June 7 she weighed 94½ lbs. only, although she was not extremely emaciated. Curiously enough, so far from losing flesh still more, she increased 4 lbs. in weight during the following week.

Murchison[1] records the case of a lady seen by him in consultation, almost identical with the present one, in which a temporary fistula was brought about by an incision into a distended gall-bladder. The common duct was blocked by a calculus, which after more than a month found its way into the duodenum, when the fistula gradually closed, and the patient entirely recovered. The amount of bile secreted averaged 40 ozs. per diem, the body-weight being 130 lbs., which agrees pretty closely with the normal amount of bile, as calculated by Carpenter from experiments on dogs, by Köllicker and Muller, Scott, Bidder and Schmidt, and Platner and Stackmann. If allowance be made for the difference in weight, the amount of bile secreted in the present case also agrees with the amount thus calculated, so that it may fairly be stated that the bile is normally secreted to the extent of 4 ozs. per 14 lbs. of body-weight during the 24 hours, or 2½ pints per diem in a man of 12 stone.

Variations in rate of flow.

The rapidity of secretion was found to vary very considerably, at different times of the day and night, and several observations were made,

[1] *Diseases of the Liver*, 3rd ed. p. 576.

extending throughout the 24 hours, to determine the relation of these variations to the meals taken by the patient. On each occasion the results were very similar and the table gives a curve Pl. XXI which represents their mean.

This variation in the rate of flow at different times of the day is contrary to the observations of v. Wittich and of Yeo and Herroun, who found no such fluctuations, but whose cases, however, were confessedly abnormal. At the same time it is certainly not due to the causes suggested by them as possibly accounting for such a variation, when occurring, if indeed it ever really did occur, namely the ingestion of abnormally large meals, or to prolonged fasts; as the meals were given, in common with those of the other patients, at regular intervals, according to the following table:

Breakfast	5 a.m.
Lunch	10 a.m.
Dinner	1 p.m.
Tea	4.30 p.m.
Supper	7.30 p.m.

As seen by reference to the curve, the rate of secretion was least at 5 a.m. when some hours had elapsed since the last meal, and greatest at 12 noon, the amount ranging between the wide limits of 14·5 c.c. in an hour and 54·5 c.c. in an equal time.

Breakfast-time, when the rate of flow was at its lowest ebb, is followed by an immediate rise for the next hour, with a secondary one two hours later still. The extensive rise about noon is curious, as the 10 o'clock lunch is not by any means a large meal, while there is no such elevation after dinner, the principal meal of the day.

This is, however probably due in part to the fact, that the cannula was usually removed about 10 a.m. for cleaning, as it tended to get somewhat choked with mucus, so presenting a resistance to the free flow of the bile unless this was attended to, and as the bile is secreted at a very low pressure a slight obstacle is sufficient to check the flow to some extent. The cleansing of the tube might be expected therefore to be followed at any rate for a time by a somewhat freer flow. It should, however, be stated that the tube never became completely blocked even when, as was the case on two or three occasions, it was not removed for 48 hours or more; while twice, when removed in the afternoon for the purpose of exploring the fistula, the cannula and also the tube were perfectly patent.

Again, shortly after supper, a primary rise occurs, followed by a still higher wave commencing two hours later on.

The act of secretion was found to consist, not of a continuous oozing, greater or less in amount according to the time of day, but on the contrary, it was noticed that there were short periods of rest, followed by a flow in a series of jerks, which appeared to be caused by waves of peristaltic contraction occasioned, doubtless, by the presence of fluid in the gall-bladder and larger bile-ducts. That this is the true explanation of the phenomenon appears likely, from the consideration that there is a plentiful supply of involuntary muscular tissue in these situations, Heidenheim indeed asserting that the layers of the muscular coat, both longitudinal and circular, are continued into even the finest branches of the bile-ducts.

Bodily movements, such as turning in bed, were also found temporarily to increase the flow, but whether there was any relation between a rise of blood-pressure in the hepatic artery and an increase in the amount of secretion could not, of course, be determined.

It is generally stated that the amount of bile secreted is increased by the presence of bile-salts in the blood going to the liver, and that therefore the amount flowing from a fistula, in which case these salts are all drained off as soon as formed, must be considerably less than the amount normally poured into the duodenum, for the reason that the greater part of the bile-salts are normally reabsorbed into the circulation, through the walls of the intestine, and so carried again to the liver, exciting by their presence a greater flow of the water of the bile. In order to test this assertion, as it seemed that in this case no bile found its way into the intestine, the patient, as mentioned above, was given 30 grains per diem of purified ox-gall, in the form of pills, for about a fortnight, after which time it was not persisted in, as the patient made great complaint of the size of the pills and of consequent difficulty in swallowing them.

At first it seemed as if this treatment had had the effect of increasing the flow, as the amount collected rose in two days from an average of 27 ozs. to 34 ozs. but it immediately commenced again to fall until the former average was again reached, while the subsequent removal of the drug occasioned no obvious alteration in the diurnal record.

Colour of the Bile.

The bile, of which so large a quantity was obtained, was not of the golden-red colour which is usually stated to be common to the liver

secretion of man and the carnivora, but was of a deep olive tint in which green certainly preponderated over the yellow. This was the case with the first few drops which oozed out into the dressings when the fistula first became established, and continued so to the end, although asepticism was apparently perfect all the time.

Yeo and Herroun make no mention of the colour of the bile in their case, but in looking over those enumerated by Murchison[1] it was found that he particularly mentions in each case that the secretion was dark green in colour.

Lauder Brunton[2] says "In man, it is, when fresh, of a golden-yellow colour, like yolk of egg, as may be seen when it is vomited," but this does not seem to be the usual experience, biliary vomit having more often a green than a yellow tint. He adds "after death the bile in the gall-bladder is generally brownish."

Now the specimens of human bile, which have been examined, other than in cases of fistula, must, at any rate as a rule, have been obtained some time after death, and probably after some considerable stay in the gall-bladder, so that it is possible that the colour varies according to the length of time that it remains in the gall-bladder, and is due to a certain amount of reduction going on there.

This theory is, moreover, capable of experimental proof. We had a number of monkeys in the laboratory, one of which happened to die, and the post-mortem examination was not made until about 12 hours afterwards, when the contents of the gall-bladder were examined and the colour of the bile was found to be a fairly deep golden-red. Next day one of the other monkeys, of the same kind as the first, was operated on, under an anæsthetic, and before the death of the animal the gall-bladder was aspirated, so as to prevent any ingress of air, when we particularly noticed that the colour of the bile withdrawn was a bright olive-green.

In a third case, which we had the opportunity of examining between five and six hours after death, the colour of the bile was found to be intermediate between that in the other two cases.

Lauder Brunton states[3] that sulphurous acid, which is a powerful deoxidizing agent, causes alkaline solutions of biliverdin, especially when warm, to become yellow; when the yellow solution is treated with nitric acid, it behaves just like a solution of bilirubin.

This experiment gives a clue to what probably takes place in the

[1] *Diseases of the Liver.* [2] *Ib.* p. 495.
[3] *Ib.* p. 498.

body, for we know that reduction can take place in the intestine, as is evidenced by the presence of stercobilin in the fæces, which is acknowledged to be a reduction product of bilirubin, and if this change be possible, there is no reason why a change of biliverdin into bilirubin should not be possible also.

The usual colour of biliary vomit is certainly a dark or olive-green, and Foster suggests that this is due to oxidation of bilirubin in the stomach, by the hydrochloric acid of the gastric juice. By treating a small quantity of bile containing bilirubin, with an excess of dilute HCl of ·2 $^{0}/_{0}$ and keeping it in an incubator at the temperature of the body, we have satisfied ourselves that this explanation is a sufficient one.

There are, however, undoubtedly instances, particularly in the case of sea-sickness, in which the vomit is of a reddish or yellowish tint, and we may suppose that when this is so, the bile in the intestine, rich in bilirubin, is suddenly regurgitated upwards, along the alimentary canal, without any lengthened stay in the stomach, the colour therefore not having undergone any change.

Again, Fagge[1] states that in long continued jaundice, the liver is found to be stained of a deep green, which he believes to be due to the oxidation of the bile during its retention in the tissues; but in our case such an explanation for the green colour of the secretion certainly could not hold, as all the bile was drained away as fast as formed, there being no possibility of its stagnating at any part of its course, since after death the gall-bladder was found to be completely atrophied, its calibre not being greater than that of the hepatic duct.

From all these facts it is, we think, a fair inference, that in man, the bile, as secreted, is normally of an olive-green colour, due to biliverdin,—bilirubin being present in comparatively small quantity, but that if it be retained in the bladder, it gradually undergoes a change in colour from green to reddish-yellow, from the reduction of the biliverdin to the stage of bilirubin, or even further to that of hydrobilirubin, when it becomes of a brownish tinge.

When poured into the intestine, these changes doubtless take place still more rapidly, so that before the pigmentary material is cast off from the economy in the fæces, it has all become converted into stercobilin, which is believed to be identical with hydrobilirubin.

Under certain circumstances, however, this change from biliverdin

[1] *Principles and practice of Medicine*, 2nd ed. p. 505.

to a lower stage of oxidation does not take place, when the products of decomposition are present in less quantity than usual, as during a dietary composed largely of fruit when the fæces are found to be of a dark green colour, which cannot be accounted for by the amount of chlorophyll taken in the food. Vegetarians, or those who live on a diet made up for the most part of fruit and vegetables, have often noted also, that under these circumstances the stools are completely odourless, a point which bears out our contention, as to the causation of the different colouration.

The reaction of the bile was tested on several occasions, and was always found to be faintly alkaline.

The specific gravity, as is always the case with fistula-bile, was very much below that commonly recorded of bladder-bile, but at the same time was somewhat higher than that observed in several cases of biliary fistula, ranging from 1·0085 to 1·105 sp. gr. and this notwithstanding that the amount poured out in the 24 hours was so very much greater than in any former record of such estimations, with the single exception of Murchison's case.

The total amount of Solids in 24 hours.

The total solids were estimated on four different occasions, in each case in a portion of the entire amount of bile passed during the previous 24 hours. On July 9th the amount was 1·423% which is more than was found by Yeo and Herroun in any of their determinations, and if the total quantity of solids present, in the whole of the bile secreted during 24 hours, be taken into consideration, the amount in this case was more than double that in theirs, the numbers being 10·863...grms. and 5·0116...grms. respectively.

Even this amount, however, is obviously much below the results arrived at in the analyses of bladder-bile, and yet we cannot consider that this comparatively small percentage of solids is due, as has been suggested, to any change in general metabolism caused by the operation which was the initial factor in the production of the fistula, nor to the lowering effect of disease, for the double reason that the amount of fluid secreted, as has been mentioned above, appears to have been quite up to the normal standard of health, as nearly as that can, with any certainty, be determined, and that, so far from there being any increase of marasmus after the operation, the woman, who, on admission to the hospital, was emaciated as the result of the suffering she had under-

gone, actually began to put on flesh immediately afterwards, on one occasion gaining as much as 4 lbs. in a single week, and on June 22nd, about two months after the operation, there is a note to the effect that Dr Bristowe told the patient that she was getting quite rosy.

We are thus driven to the conclusion that the difference must be accounted for in the way suggested by Schiff and others; that there is normally a bile circulation going on in the body, a large quantity, at any rate of the bile-salts which pass into the intestine, being reabsorbed and yet again secreted. This view is supported by the fact that cholalic acid is found in very small quantity only in the fæces. Such a circulation of the bile-salts would obviously be impossible in a case like the present.

As was mentioned above, it was found that on treating the patient with pills of concentrated ox-gall, a slightly increased flow was, for a time, obtained, but if the question is a mere one of circulation, it is obvious that the amount given (10 grs. *ter die*) was quite insufficient to raise, in any appreciable degree, the percentage of solids in the bile.

Another point in favour of the view advocated, is found in the fact that the liver was apparently perfectly healthy, no evidence of disease being found after death. Consequently it is to be presumed that it was competent to perform its normal functions; a proof that this was so being, that both urea and uric acid were found in normal quantity in the urine, and it is thus fair to suppose that the bile-salts were also manufactured in normal amount, the reason for their low percentage at any one time being that they were drained off in the bile, as soon as formed, instead of, at any rate in part, finding their way back to the liver.

It has been found by Bidder and Schmidt[1] that the percentage of solids of the freshly secreted bile from a temporary fistula fell from 6·6 % to 3·8 % in two hours, and much stress has been laid on this observation, and others corroborating it, as disproving the idea of a bile-circulation, but it seems to us rather to support it than otherwise, for it is hardly likely that in so short a time the functional activity of the liver would be destroyed to any great extent, whereas the impossibility of any of the amount of bile-salts formed doing duty a second time would soon reduce their apparent production to the extremely low and nearly dead level observed in a permanent fistula, we having found the amount to vary within very small extremes during weeks of observation.

[1] *Die Verdauungssäfte, &c.*

It seems probable that the large difference in the amount of solids between bladder-bile and that obtained from a fistula, may be caused, to even a greater extent, by a considerable portion of the fluid, originally secreted, being absorbed during its sojourn in the gall-bladder, for although Hoppe-Seyler[1] objects that this theory requires the assumption that the mucous membrane of the bladder has a special absorbent and selective property, still it has on many occasions, as in Murchison's case, quoted above, been noticed that where the cystic or common ducts have become blocked in any way, the gall-bladder, when opened at a later period, has always contained a fluid in which neither bile-salts nor pigment were to be found, having been evidently replaced, after absorption by the mucous secretion of the lining membrane of the gall-bladder. In the present case there was a blocking of the common duct, but the cystic duct was patent, and yet the bladder contained a fluid which had none of the characters of bile.

It also seems likely that simple physical diffusion may account for the removal of a portion of the water, since the specific gravity of the bile is very much lower than that of the blood, and we know that the walls of the gall-bladder are very richly supplied, both with lymphatics and blood-vessels, especially near the surface of the mucous membrane.

Quantitative Estimation of the Solid Constituents.

The determination of the solids was made by the method suggested by Dr Charles[2], which is based on that of Gorup-Besanez.

Twenty grams of the bile were weighed out in a capsule, and after evaporating to dryness, the residue was heated for some time at 110° to ensure complete dessication. The weight on cooling gave the total solids.

The dry mass was then exhausted with absolute alcohol, and the insoluble residue, consisting of mucin, a little pigment, and insoluble salts, was dried and weighed as before. The salts were obtained quite free from organic matter by careful incineration of this residue and were then weighed.

The salts of the biliary acids were precipitated in the crystalline form by evaporating the alcoholic extract to small volume, adding excess of ether and allowing the mixture to stand for several days, this

[1] *Physiol. Chem.* § 310, 1878.
[2] *Physiol. Chem.* p. 204.

process being repeated five or six times. They were then dissolved in a little alcohol, evaporated in a capsule, dried and weighed.

Cholesterin, fat, and lecithin were obtained by distilling the ethereal extract and well drying the residue over sulphuric acid, but their quantity was so small that no attempt was made to determine their several amounts.

A number of estimations were made with specimens of bile passed on different days. These all agreed very closely, and chiefly differ from that of Yeo and Herroun[1] in the amount of bile-salts present, which was much greater in the present case than in theirs.

The following table gives the percentage composition of a specimen of the bile, which contained 1·423 % of solids, as compared with Frerich's analysis of supposed normal bile.

	Ourselves	Frerich
Sodium glycocholate Sodium taurocholate	·628	9·14
Cholesterin, Lecithin and Fat	·099	1·18
Mucus	·1725	2·98
Pigment	·0725	
Inorganic Salts	·451	·78
	1·423	14·08
Water (by difference)	98·577	85·92
	100·000	100·00

The Rôle of the Bile in Digestion.

The part played by the bile, in the various processes of digestion taking place in the intestine, is usually divided under the following heads:—

1. The neutralization of the acid chyme and the precipitation of the parapeptones, and with them the pepsin.
2. The emulsification of fats.
3. [2] It influences pancreatic amylotic digestion.
4. It aids absorption, particularly of fats.
5. It excites peristaltic action, and the abundant supply of water and mucus, moistens and lubricates the fæces, and so facilitates their evacuation.

[1] This *Journal*, Vol. v. p. 122.

[2] Martin and Dawson Williams, *Proceedings of Physiological Soc.* 1888.

6. It acts as an antiseptic.

As the case under consideration throws some light on these points, it will be well to consider them, more especially the last, a little in detail.

Curiously enough, throughout the whole course of the illness the patient appears never to have suffered from constipation, the fæces being of the colour and consistence of thin mortar.

The whole of the excreta, passed during the 24 hours, was collected and examined on two or three different occasions, when it was found, as might have been expected, that they contained a considerable amount of fats, once rather more than 18 %, showing that, in the absence of the bile, the pancreatic juice is not able to complete their digestion. To test this matter still further, Dr Bristowe ordered half a pound of butter to be taken with the meals in the course of the day, but in consequence of the intense nausea and vomiting which ensued it was not persisted in.

The amount of undigested proteid material, however, was insignificant, so that the pancreatic juice was apparently able to deal satisfactorily with any proteids which had escaped the action of the stomach. It appears that the pancreas must have been stimulated by the presence of the acid chyme in the duodenum, to pour out a more abundant secretion than usual, and since, when this is the case with any gland, the percentage of inorganic salts remains about the same, or may even be increased, the sodium carbonate would be sufficient in quantity to neutralize the products of gastric digestion (1 c.c. of pancreatic juice neutralizing ·4 c.c. of the gastric secretion), and so allow its special ferment to act normally on the proteids present.

There were never any symptoms of dyspepsia, with the exception of the special occasion referred to above, and the patient had a voracious appetite, often complaining that she had not sufficient food. This symptom is interesting, since it has been found experimentally, that dogs, in which a biliary fistula has been formed, require a larger quantity of food to maintain their nutrition than before, more proteid material having to be broken up to supply the fat necessary for the organism.

Although pancreatic digestion was obviously interfered with, as far as regards the assimilation of fats, the patient's health did not suffer on this account, but on the contrary, as has been stated, she gained in strength and in actual body-weight during the time she was in the hospital.

The Bile as an Antiseptic.

The statement that bile acts as an antiseptic, is to be found in all the text books, and apparently depends on the fact that when the bile is in any way prevented from entering the duodenum, as in the various forms of jaundice, or when it is deficient in quantity, the evacuations are often extremely fetid, from the contents of the intestine undergoing changes different to those which occur in a normal condition.

Out of the body its presence hinders various putrefactive processes[1] especially, as has been remarked on numerous occasions, in the liver itself; for this organ, particularly if obtained post-mortem from a case of obstructive jaundice, so that it contains an excess of bile, may be kept for a considerable time, even in warm weather, without undergoing any putrefactive change. It should however be remembered that other organs resist this process even more successfully, since according to Taylor, Guy and Ferrier, Husband, and other writers on forensic medicine, the uterus particularly can be recognized long after all the other organs have disappeared.

It is also noteworthy, that bacteria abound and thrive in the intestine, and that bile itself will putrefy if left for a time exposed to the air.

Dr Sherrington, moreover, had made the observation that Koch's cholera bacillus, the spirillum from Indian cultures, Eberth's and Ribbert's bacilli, all grew vigorously, when inoculated into sterilized ox-bile, and into nutrient gelatine to which had been added an equal quantity of ox-bile. It was therefore determined to carry out some experiments, with the object of ascertaining whether the universally accepted view as to the antiseptic powers of the bile had, in the case of man, any foundation in fact or not.

To this end a number of tubes of bile were sterilized, and afterwards inoculated with organisms of various kinds, both pathogenic and otherwise, but this method was subsequently abandoned for one that appeared to be capable of affording more accurate results. It seemed possible that as the bile contains so small a quantity of organic material, growth might not be possible, even supposing that no actual restraining influence were present, while at the same time a fluid medium is not usually at all satisfactory for bacteriological work.

With the object of doing away with these objections, and at the same time obtaining control cultivations, tubes containing nutrient

[1] Foster's *Physiology*, 4th ed. p. 250.

gelatine were used. Some were merely filled with jelly prepared in the usual manner, while in others a portion of the water used was replaced by varying but definite amounts of bile obtained from the fistula, 30 °/₀ and 60 °/₀ of bile being the quantities generally used. Some tubes however were used which contained a jelly made entirely with bile without the addition of any water beyond that contained in the secretion. The amount of gelatine used in every case was 10 °/₀.

The great difficulty in preparing this nutrient bile-gelatine was to obtain it sufficiently clear, but this was accomplished by filtering through doubled paper in the hot funnel, if necessary several times over, great care being taken not to allow the temperature to rise any higher than was necessary.

The resulting material was of a light greenish-brown colour, and in successful cases, quite transparent.

Seeing that the bile obtained from this case was evidently very much more dilute than that which is normally poured into the intestine, we finally made use only of the jelly made from the fistula-bile without any further addition of water.

Some of the results of the inoculations, which in each case were made both in bile-gelatine and also in ordinary nutrient jelly as a control experiment, are shown in the following table.

No. of tube	Organism inoculated	Medium	Results
39	Anthrax	Bile-gelatine	slight growth in 2 *days*
40	Anthrax	Gelatine	rather less in 2 *days*
3	Anthrax	Bile-gelatine	complete liquefaction in 3 *weeks*
4	Anthrax	Gelatine	ditto in 5 *weeks*
49	Finkler and Prior's spirillum	Bile-gelatine	good growth in 6 *days*
48	Finkler and Prior's spirillum	Gelatine	good, but slightly less in 6 *days*
28	Rabbit Septicæmia	Bile-gelatine	fair growth in 6 *days*
29	Rabbit Septicæmia	Gelatine	same amount of growth in 2 *days*
36	Bacterium prodigiosum	Bile-gelatine	fair growth in 3 *days*
37	Bacterium prodigiosum	Gelatine	very slight in 3 *days*
50	Decomposing serum	Bile-gelatine	good growth in 7 *days*
51	Decomposing serum	Gelatine	good growth in 24 *hours*
44	Putrid meat infusion	Bile-gelatine	fair surface growth in 3 *days*
43	Putrid meat infusion	Gelatine	good growth in 3 *days*
35	Coccus from Bile	Bile-gelatine	very slight growth in 5 *days*
34	Coccus from Bile	Gelatine	good growth in 2 *days*

In not a single instance was growth altogether prevented by the presence of the bile.

Similar results were obtained with bile-gelatine, made from human bile, obtained from the gall-bladder post-mortem, although, with equal percentages, the results were somewhat more marked, this being doubtless due to the greater concentration of the bile in this case.

Experiments were also carried out with nutrient gelatine in which ox-gall was substituted for the human bile previously employed, with the object of comparing their action on bacteria, and in this case again the results were found to be the same.

It is usually stated, that the so-called antiseptic power of the bile is due to the presence of the bile-salts, and since bile undoubtedly does possess a slight restraining effect on ordinary putrefactive organisms, as is shown by the table, experiments are being conducted with the object of determining whether this be so, and if it be, whether glycocholic or taurocholic acid is the more important in this respect, but the results must be reserved for a future communication.

Although the bile evidently remained, from first to last, free from the slightest sign of decomposition, still, on inoculating a drop of it into nutrient jelly, with every precaution for preventing ingress of germs, a growth was obtained in each case, the nature of which has not yet been accurately determined. Consequently spores must have been present in it, which may have originally made their way from the intestine, but which were rendered dormant, although not killed, by the slight anti-putrefactive power of the bile.

Tubes were also inoculated with the contents of the gall-bladder of guinea-pigs, obtained with the strictest antiseptic precautions, and in these cases also, a slight growth resulted along the track of the needle after some lapse of time.

These experiments show most conclusively that the bile cannot be regarded as an antiseptic in the strict sense of the term, although it doubtless has some effect although not a very powerful one in preventing ordinary putrefactive processes.

The Relation of the Bile and Urinary Pigments.

It is a well-known fact, that in old blood-clots, particularly in the brain, crystals of a brown colour, and consisting of a body termed hæmatoidin, not unfrequently occur. This body is believed to be identical

with bilirubin, the colouring matter of the bile, and also with iron-free-hæmatin, which may be obtained experimentally from the hæmoglobin of the blood.

Bilirubin, again, when reduced by means of sodium amalgam, gives rise to hydrobilirubin, which is probably identical with urobilin, the most important of the urinary pigments, since they both present precisely similar spectroscopic appearances, and it has been suggested that this change normally takes place in the alimentary canal; a portion, at any rate, of the pigment of the bile, when poured into the duodenum, becoming converted through the agency of hydrogen or other reducing agents there present[1] into hydrobilirubin, which is then again absorbed through the walls of the intestine, carried again to the liver, so into the general circulation and finally to the kidneys, where it is eliminated with the urine.

This theory of reduction in the intestine may perhaps be sufficient to account for the formation of stercobilin, the fæcal pigment, which is also considered to be a reduction product of bilirubin, but it certainly does not appear to be in accordance with facts observed in the case now under discussion, as far as the urobilin is concerned.

The urine was repeatedly examined, and the amount of its chief constituents carefully determined. Except on a few occasions, when the patient was not so well as usual, it was perfectly clear when passed, containing no albumen, and being usually somewhat high-coloured even when no trace of bile-pigment was present. Indican was conspicuous by its absence. The following table, showing the composition of the urine passed during 24 hours on the 8th and 9th of July, agrees very closely with the determinations on other days.

Total amount 905 c.c.
„ urea 23·5 grms.
„ uric acid ·209 grms.
Specific gravity 1018, reaction acid.

This amount (31 ozs.) however does not account for all that was passed, as some was unavoidably lost with the fæces.

For several weeks before the date of this analysis, the whole of the bile had drained off from the fistula as soon as formed, it having been proved by repeated examinations that it was completely absent from the fæces and urine.

Since then, no bile found its way into the intestine; it appears

[1] *Ib.* p. 499.

obvious that in this case the urobilin could not be formed from bile-pigment, in the manner usually stated, and yet the urinary pigment was by no means deficient in quantity.

A plausible explanation of these facts seems to be, that biliverdin, bilirubin, and hydrobilirubin, are all formed in the liver, either from hæmoglobin or some closely allied body; not the one from the other, but by a slight divergence of metabolism, such as must take place in the simultaneous formation of urea, and of uric acid, a less oxidized body; the liver cells by virtue of an inherent selective power retaining the bilirubin or biliverdin and then extruding it into the primitive bile-ducts, while the hydrobilirubin finds its way into the circulation, and is passed out of the system as urobilin by the renal epithelium, which has of itself no power of converting the one substance into the other, as is proved by the fact, that, in cases of obstructive jaundice, the excess of bile-pigment, which passes into the circulation, appears in the urine as such and not as urobilin.

So also, when solutions of hæmoglobin, or of any substances which will liberate hæmoglobin from the corpuscles, such as bile-acids or æther, are injected into the vessels, a quantity of bile-pigment is often formed, a portion of which appears in that form in the urine.

The blood, moreover, is known to possess no reducing power, so that we are brought back to the consideration, that the birthplace of the urobilin, like that of the bile-pigments, must be the liver.

If this be the case, it is not astonishing that the urinary pigment was not apparently decreased in the present case, since there is no reason for supposing that the functions of the liver were in any way suspended, the analysis of the urine, given above, showing that both urea and uric acid were present in about the normal quantity, while it should also be remembered that the liver, when examined after death, appeared quite healthy.

It may be objected that urobilin is not the only colouring matter of the urine, and although this is doubtless the case, the matter is still sub judice and requires further investigation. However, McMunn[1] states that urobilin is constantly present in every specimen of human urine in health, and that when this fluid is high-coloured, the absorption band of urobilin at *F* is dark, while the band is feeble when the colour is light, thus showing that differences in the tint of the urine certainly correspond to similar differences in the amount of urobilin present.

[1] *The Spectroscope in Medicine*, p. 167.

Summary.

As the result of our observations, we have arrived at the following conclusions:—

1. The normal amount of bile secreted by the liver is about 48 ozs. or 2½ pints per diem in a man of 12 stone.

2. The rate of flow varies in accordance with the time of ingestion of food; there being usually a rise between one and two hours after a meal. The secretion, moreover, is not a continuous one, the bile being extruded into the gall-bladder by repeated peristaltic contractions of the bile-ducts.

3. The colour of the bile in man is probably always of an olive-green, biliverdin, and not bilirubin, being the pigment which is present in greatest quantity.

4. The amount of solids present is normally about 10 %, this percentage being kept up by a continuous reabsorption of bile-salts from the intestine, following on their secretion; the specific gravity of the bile falling when it is drained away as fast as formed, as occurs in a fistula. The amount may also be increased, after the bile is first formed, by absorption of water through the walls of the gall-bladder.

5. Bile is necessary to the assimilation of fats, but not absolutely so, although doubtless of use, for that of proteids. Its purgative action is more than doubtful.

6. The bile has no true antiseptic action, although it is able to a small extent to control putrefactive changes.

7. Stercobilin probably is formed by reduction of the bile-pigments in the intestine, but urobilin appears to be formed in the liver, together with the bile-pigments, by a slight change of metabolism.

EXPLANATION OF PLATE XXI.

The curve, which shows the variations in the flow of bile from the fistula, during 24 hours, was built up by direct measurement, every hour throughout the day and night, of the amount secreted during the preceding hour. It does not represent the rate of flow during any one day, but, as stated in the text, is the mean of several observations.

CEREBRO-SPINAL FLUID. By W. D. HALLIBURTON, M.D., B.Sc., *Assistant Professor of Physiology, University College, London.*

(*From the Physiological Laboratory, University College, London.*)

During the last few years, I have had many opportunities of examining various pathological fluids, removed by paracentesis, and among these I have been able to obtain a few specimens of cerebro-spinal fluid, removed either from meningocœles, or from cases of hydrocephalus.

In the present paper I shall give the results of my examination of these last mentioned fluids, reserving for a later contribution an account of the fluids removed from serous cavities. It seems to be advisable to make this separation, because in several points cerebro-spinal fluid differs very markedly from the fluids found in the serous cavities. The fluids in the serous membranes are in health present in very small quantity, and our knowledge concerning them is derived almost entirely from cases of disease in which owing either to a disturbance of the vascular system, or a change in the blood itself (anæmia), they are increased in quantity. An examination of these fluids shows that like normal lymph they are transudations from the blood, and may be briefly described as diluted blood plasma.

But with cerebro-spinal fluid it is different; the fluid in the cerebro-spinal canal is in direct communication with that in the sub-arachnoid cavity by means of the foramen of Magendie. The situation of the fluid in question is thus anatomically very different from that of the fluids contained in the cavities of serous membranes. The arachnoid membrane has certainly by some been described as a serous membrane, but from the point of view both of anatomy, and of embryology, there is no justification for classifying it with such membranes. The fluid also appears to be under different mechanical relations to the parts which it bathes; there is not merely sufficient to moisten the membrane, but judging from experiments on animals, in which the spinal cord is exposed, it seems to

be present in sufficient quantity to exert a considerable amount of pressure. There have also never been described any stomata such as occur in true serous membranes, by which the fluid is brought into direct communication with the interior of the vascular system, *via* the lymphatic channels; and lastly, the fluid itself is not merely dilute lymph, but has certain special characteristics of its own; it will be the object of the remainder of this paper to put this last point more fully in reference to my own experiments; I think we shall see in conclusion, that C. Schmidt was justified in classifying cerebro-spinal fluid not with transudations, but rather with secretions. This conclusion will however be based upon different grounds from those of Schmidt. I shall not enter at all into the question whether the epithelium of the cerebro-spinal canal is concerned in elaborating or secreting the fluid contained in that cavity.

I am indebted for my specimens chiefly to Mr R. W. Parker; these were removed from cases occurring in his practice at the East London Hospital for Children. I have also received a few specimens from Dr Ringer, Mr A. E. Barker and Mr V. Horsley from cases in University College Hospital, from Dr Penrose from cases at the Hospital for Sick Children, Great Ormond Street and from Mr S. G. Shattock, to all of these gentlemen, I beg to offer my sincerest thanks for the assistance they have thus given me.

The first three specimens[1] I examined were from cases of spina bifida; these I chiefly devoted to determining the proportions of the total solids and water, and of the chief classes of solids present.

CASE 1. Female æt. 19 years. Fluid faintly opalescent, faintly alkaline; specific gravity 1007.

	Parts per 1000.
Water	989·75
Solids	10·25
Proteids[2]	0·842
Reducing substance (estimated as dextrose)	0·002 (approximate)
Extractives and soluble salts	9·406
Insoluble salts	0·218

[1] The fluid from Case 1 was sent to me by Mr S. G. Shattock, and from Cases 2 and 3 by Mr R. W. Parker. The results of these three analyses have been already published in the Report of the Spina Bifida Committee, Vol. XVII., *Clinical Society's Transactions*, 1885.

[2] Estimated by weighing the precipitate produced by adding a large excess of absolute alcohol.

CASE 2. Female æt. 11 days. Lumbo-sacral spina bifida. First tapping.

Fluid perfectly clear; faint yellow tinge; reaction slightly alkaline.

	Parts per 1000.
Water	919·877
Solids	10·123
Proteids	1·602
Reducing substance	traces
Extractives	0·631
Soluble salts	7·544
Insoluble salts	0·346

CASE 3. Male æt. 13 weeks. Fourth tapping; iodine had been injected on three previous occasions.

Fluid perfectly clear, colourless, faintly alkaline.

	Parts per 1000.	
Water	991·658	
Solids	8·342	
Proteids	0·199	
Reducing substance	0·165	(reckoned as dextrose)
Other extractives	2·863	
Soluble salts	4·776	
Insoluble salts	0·339	

Tabular statements of this kind however do not show anything characteristic of the fluid examined. They show

(1) A very small percentage of proteids.

(2) A proportion of inorganic salts approximately equal to that in the blood, lymph, and transudations generally.

(3) A small proportion of a substance which reduces alkaline solutions of copper salts like sugar.

But all these characteristics might be equally well possessed by a dilute transudation such as occurs in œdema of the subcutaneous tissues; in many cases of subcutaneous œdema, there is often a small percentage of sugar as in lymph generally[1].

[1] For sugar in lymph see v. Mering, *Ludwig's Arbeiten*, 1877; in peritoneal fluid, Cobrat, *Lyon méd.* 1875, No. 15; Lépine, *Gaz. méd. de Paris*, 1876, p. 123; Quincke, *Berl. med. Wochensch.* 1876, No. 38; Moscatelli, *Zeitsch. für physiol. Chem.* XIII. p. 202 (in many of these cases the fluid was obtained from cases of liver cirrhosis, which is occasionally accompanied with a mild degree of glycosuria); in pleuritic fluid, Eichhorst, *Zeitsch. f. klin. Med.* III. p. 537; in œdema fluid, A. Rosenbach, *Breslauer arztl. Zeit.* 1885, No. 5. In all these cases it seems to be really sugar that is present; that is a

The peculiarity of cerebro-spinal fluid lies in the quality not the quantity of proteids that are present, and in the fact that the reducing substance present is not sugar, but something else.

Before proceeding however to give my final conclusions, it will be convenient to state briefly the experiments performed with each of the specimens in the order in which they have come under my notice.

CASE 1. Quantitative analysis already given.

Proteids. Boiling the liquid after rendering it faintly acid produced a small amount of flocculent precipitate. Saturating another portion with magnesium sulphate produced a precipitate: the precipitate was washed, collected and redissolved; the solution was opalescent: on heating it, coagulation occurred at 75° C.

On filtering off the precipitate produced by magnesium sulphate, a clear filtrate was obtained; on boiling this, after the addition of a few drops of 2°/₀ acetic acid, it remained clear: nitric acid gave a very faint precipitate, and on boiling this a yellow solution formed, which was turned orange by ammonia. Copper sulphate and potash gave a colour which could not be well observed because of the precipitate of magnesia produced on adding potash.

Conclusions. The greater part of the proteid consisted of a globulin apparently identical with serum globulin; a small quantity of another proteid was present the reactions of which were very puzzling to me at the time I made the analysis, as I was not then familiar with the work of Kühne and Chittenden on albumoses. Judging however from the few tests I tried, the reactions seem to be those of deutero-albumose (i.e. a proteid not coagulated by heat, not precipitated by magnesium sulphate, precipitable by nitric acid in the cold in the presence of salt, and dissolving up on heating).

CASE 2. The amount of fluid sent me was very small, and beyond the fact that saturation with magnesium sulphate produced a precipitate, my notes show nothing more than the quantitative analysis already given.

CASE 3. Quantitative analysis already given.

Proteids. The quantity of proteids was exceedingly small in comparison with the other cases: and this was the case in spite of the previous injection of iodine into the sac on three occasions.

[This should be contrasted with Cases 9 and 12 in which the amount of proteid increased after repeated tappings. It is also in contrast to the

substance which not merely reduces cupric salts, but undergoes the alcoholic fermentation on the addition of yeast, etc. In cerebro-spinal fluid we shall see presently the reducing substance is not sugar.

increased quantity of reducing substance; that the reducing substance increases with successive tappings has been noted by previous observers.]

The chief point of interest with regard to the proteids was that saturation with magnesium sulphate produced a precipitate, on the removal of which the filtrate was entirely free from proteids On redissolving the precipitate by the addition of water it was found that it coagulated at 75° C., and after filtering off the heat coagulum, no proteid remained in solution.

Conclusions. The proteid present consisted entirely of a globulin, which is apparently identical with serum globulin.

CASE 4. Fluid removed from spina bifida by Mr A. E. Barker.

A small quantity only was received, about five cubic centimetres in all. It was clear and colourless like distilled water; and slightly alkaline in reaction.

It was saturated with magnesium sulphate, and a precipitate was thus produced: this was collected on a small filter, washed with saturated solution of magnesium sulphate, and redissolved by the addition of water: the properties of this solution were as follows:—

(1) On heating to 75° C. after acidifying with weak acetic acid, there was no distinct precipitate, only a cloudiness.

(2) Nitric acid caused a precipitate in the cold which disappeared on heating, and reappeared on cooling.

The filtrate obtained by filtering off the magnesium sulphate precipitate was clear and colourless, and remained so on boiling, after acidifying with acetic acid. Nitric acid gives a precipitate which disappeared on heating, and reappeared on cooling: the xanthoproteic reaction gave an intense orange colour.

Conclusions. Although I had so small a quantity of fluid at my disposal, the typical albumose reaction with nitric acid was so manifest, that it was from the examination of this specimen that the idea that albumoses are present in cerebro-spinal fluid was first suggested to me. The above reactions would of course be insufficient in themselves from which to draw conclusions; they require to be confirmed and corrected by additional tests: it was therefore necessary that I should perform such confirmatory tests in fluids of which I had a larger supply, and judging from these later experiments, I should be inclined to say that in this particular fluid, the proteids present were: globulin—a mere trace; a primary albumose, i.e. either proto- or hetero-albumose; and deutero-albumose.

CASE 5. The fluid was removed from the ventricles of the brain post-

mortem from a case of myxœdema[1]. About 15 c.c. were obtained in all, mixed with a small quantity of blood. There was however no formation of fibrin in spite of this. The corpuscles were allowed to subside, and the supernatant fluid was then seen to be slightly cloudy. Its reaction was faintly alkaline; it gave no precipitate with acetic acid; mucin was therefore absent.

Proteids. An examination of the kinds of proteids present was of little value on account of the admixture with blood. The result obtained however was this; the precipitate produced by saturation with magnesium sulphate contained a globulin coagulated by heat at 75° C. and an albumose. The precipitate produced by saturation with magnesium sulphate was removed by filtration, and the only proteid in the filtrate was serum albumin.

Conclusions. These are as follows:—(1) Fibrinogen was absent, that is admixture with blood produced no formation of fibrin. (2) Mucin was absent; this observation is of some importance as mucin has been described in the blood and transudations in cases of myxœdema. (3) In spite of the admixture with blood the typical nitric acid reaction of an albumose was obtained.

CASE 6. This was a case of heart disease[2], in which post-mortem an excess of fluid in the sub-arachnoid cavity was found. In removing it it was contaminated with rather a larger quantity of blood than in Case 5. There was however no formation of fibrin. No further observations were made.

CASE 7. Case of acute hydrocephalus[3]. This occurred in a child æt. 5 months and the fluid was twice removed during life by paracentesis from the cerebral ventricles. The fluid, about 4 oz., I received was from the first tapping.

The fluid was clear, straw coloured, and faintly alkaline.

It contained a small shrunken clot of fibrin; a microscopic examination of this clot showed it to consist of fibrin entangling a number of leucocytes: no red corpuscles.

Proteids. The presence of the clot indicated that fibrinogen and fibrin ferment (derived from the leucocytes) must have been present in the liquid.

The clot was removed, and the total percentage of proteids present in the liquid was found to be 0·65. This was estimated by adding excess of alcohol to a known weight of the fluid; the precipitate produced was collected,

[1] The case occurred in Univ. Coll. Hospital under the care of the late Dr Wilson Fox; the post-mortem examination was made by Mr Horsley to whom I am indebted for the fluid.

[2] Under the care of Dr Ringer, Univ. Coll. Hospital.

[3] Under the care of Dr Sturges, Hospital for Sick Children, Great Ormond Street. I am indebted to Dr Penrose for the fluid.

washed, dried, and weighed on a filter of known weight. The precipitate produced by the alcohol was colourless; the alcohol itself extracted the yellow colouring matter originally present, as it does the pigment present in serum. The alcoholic extract after a few days' exposure to light became bleached as such solutions of serum-lutein do.

Another portion was saturated with magnesium sulphate; an abundant precipitate was produced. The precipitate was washed on the filter with saturated solution of magnesium sulphate, and dissolved by the addition of water: an opalescent solution being the result. This solution was heated, a few drops of 2 per cent. acetic acid being first added: at 75° C. a precipitate formed (serum globulin): this was removed by filtration. The filtrate gave no further precipitate on heating. Nitric acid gave a precipitate which disappeared on heating and reappeared on cooling. Copper sulphate gave a precipitate soluble in excess. Copper sulphate and caustic potash gave a pink colour (biuret reaction). Perchloride of mercury and basic acetate of lead also gave precipitates. The liquid from which the precipitate produced by saturation with magnesium sulphate had been removed by filtration gave a precipitate on heating to 73° C. after acidifying; this was filtered off; a small quantity of proteid still remained in solution which was precipitated by boiling the liquid; on filtering this off, the filtrate was found to be free from proteid.

A portion of the original liquid was treated with ferric acetate, and the filtrate found to contain no proteid (i.e. peptone was absent).

Reducing substance. The original liquid contained a small quantity of a substance which reduced Fehling's solution. The alcoholic extract also contained the reducing substance. The phenyl-hydrazine test for sugar was then applied: this is a test recently introduced by v. Jaksch[1]. On the addition of a solution of phenyl-hydrazine hydrochloride containing a small quantity of sodium acetate to a solution of sugar, a yellow precipitate of needles of phenyl-glucazone, occurring both singly and in bundles, forms in a few minutes. I tried this test several times, but in all cases with a negative result. I then took the remainder of the fluid at my disposal, which was a little over an ounce in quantity, precipitated the proteids by excess of alcohol and filtered off the precipitate; evaporated the alcoholic filtrate to dryness at 40° C.: took up the residue with water, and evaporated to dryness again; a crystalline residue was obtained, which on microscopic examination showed a number of crystals of sodium chloride, and in addition a number of feathery laminæ, exactly similar to those obtained by evaporating to dryness an aqueous solution of benzoic acid. The crystalline residue was turned dark green, and finally black with ferric chloride; it also turned yellow on heating it to 50° C.

[1] *Zeit. klin. Med.* Vol. XI. p. 20.

Conclusions. The liquid contained in comparison with normal cerebro-spinal fluid a very high percentage of proteids: it is a general rule with effusions, that inflammation increases the percentage of proteids. The fluid moreover was not normal cerebro-spinal fluid, inasmuch as it contained a number of leucocytes, and fibrinogen resulting in the formation of a clot; serum globulin and serum albumin. In other words it resembled an ordinary exudation such as one finds in the serous sacs, and doubtless the fluid present was in great measure an effusion from the blood-vessels. In spite of this, however, proto-albumose was very distinctly present; and so also was the reducing substance, which the phenyl-hydrazine test showed was not sugar. The other tests above given leave its nature uncertain, pointing however in the direction of an aromatic substance. Peptone was absent.

CASE 8. Cerebral meningocœle in a child æt. 9 months.

The fluid I received was from the second tapping[1]. Its specific gravity was 1007; its reaction neutral or faintly alkaline: it was all but colourless. It contained no clot.

Proteids. It gave the nitric acid test for albumose without further treatment. On acidifying and heating to 75° C. there was a faint cloud: after repeated filtration, a clear filtrate was obtained; this remained clear on boiling; it gave the albumose test with nitric acid, and also the biuret reaction.

A portion was saturated with ammonium sulphate and filtered; the filtrate was proteid-free.

Another portion was saturated with sodio-magnesium sulphate and filtered: the filtrate was proteid-free.

A third portion was saturated with magnesium sulphate, a precipitate was produced and filtered off. The filtrate gave an exceedingly faint xantho-proteic reaction; this was the only evidence obtained of the presence of proteid: the yellow tinge was however so slight, that it was necessary to compare it with distilled water to make certain there was any colouration at all. In other words the filtrate was practically proteid-free.

Reducing substance. This was present in such small quantities that it was necessary to concentrate some of the liquid to about a quarter of its original bulk before any reduction could be observed with Fehling's solution.

Conclusions. The proteids present were a small quantity of globulin coagulating at a temperature of 75° C., and proto-albumose. Fibrinogen, serum albumin, and peptone were absent. A reducing substance was present in traces.

[1] I am indebted to Dr Penrose for this specimen.

CASE 9. Case of spina bifida in a child (F.) æt. 2 months[1]. First tapping (June 1, 1887). Fluid faintly alkaline, clear, colourless.

Proteids. A portion of the fluid was boiled after acidifying with weak acetic acid; a small heat coagulum formed. This was filtered off: the filtrate gave a precipitate with nitric acid in the cold, soluble on heating, and reappearing when the liquid was cooled. Copper sulphate gave a precipitate, which dissolved on the addition of potash forming a pink solution.

Another portion was saturated with ammonium sulphate: the precipitate so produced was filtered off, and the filtrate was free from proteid.

Another portion was saturated with magnesium sulphate, and the precipitate so produced filtered off. This precipitate was washed with saturated solution of magnesium sulphate, and dissolved by the addition of water. The solution so formed was opalescent and gave a precipitate on heating to 75° C. This was filtered off, and the filtrate on boiling gave no further precipitate. There was however proteid in solution which gave the albumose reaction with nitric acid and the biuret reaction. The remainder of this solution was placed in a dialyser of parchment paper, and the salts removed by dialysing into distilled water for a week. The albumose was still in solution at the end of this time.

The liquid, from which the precipitate produced by saturation with magnesium sulphate had been removed by filtration, was again agitated with excess of magnesium sulphate crystals to make sure of the precipitation of all globulin etc. and then filtered. The filtrate contained a small quantity of an albumin which was coagulated at a temperature of 73° C., and was therefore probably identical with serum albumin. This heat coagulum was filtered off; the filtrate was found to be proteid-free.

Another portion of the original cerebro-spinal fluid was mixed with blood serum and kept in a warm bath at 35°—40° C. for 24 hours. There was no formation of fibrin.

Some magnesium sulphate blood plasma of the sheep was diluted with six times its volume of the cerebro-spinal fluid; but no coagulation occurred even after the lapse of 24 hours; another specimen of the same salted plasma which was diluted to the same extent with a solution of fibrin ferment coagulated in about half an hour.

Reducing substance; a substance which reduced Fehling's solution was present in small quantities but was not further investigated.

Conclusions. The proteids present were a globulin coagulating at 75° C.: an albumin coagulating at 73° C. and proto-albumose. Fibrinogen, fibrin ferment (cell globulin) and peptone were all absent.

[1] Received from Mr Parker.

CASE 9*. Same case as preceding. Second tapping June 17, 1887; iodine had been injected after the first tapping. The fluid was examined in exactly the same manner as that removed by the first tapping.

The total proteids were increased in amount; these were not estimated quantitatively, but the increase was seen by the fact that saturation with ammonium sulphate produced a heavier precipitate than it did in that removed by the first operation[1]. Otherwise the results were exactly the same in the two fluids.

CASE 10. Case of chronic hydrocephalus[2] in a child (H. R.). The fluid removed by paracentesis was alkaline and colourless.

It contained a small amount of a substance which reduced Fehling's solution. This was not further investigated.

Proteids. Boiling after acidification with acetic acid produced a small amount of precipitate which, since it was not lessened by the addition of a drop of hydrochloric acid as is the precipitate produced on heating hetero-albumose, consisted of coagulated proteid. The heat coagulum having been filtered off the filtrate gave the nitric acid reaction for albumose and the biuret reaction.

A portion was saturated with ammonium sulphate and filtered; the filtrate was then rendered faintly acid with acetic acid and shaken for three hours with excess of ammonium sulphate crystals, and again filtered: the filtrate was shaken for a third period of three hours with more crystals of the salt[3], and again filtered. The last filtrate gave no precipitate with nitric acid; it gave a well marked xanthoproteic reaction, a well marked biuret reaction, and a precipitate with tannic acid. That is true peptone was present.

Another portion was saturated with magnesium sulphate; the precipitate so produced was washed and redissolved in the usual way, and the solution so obtained was found to contain a globulin coagulating at 75° C.; on filtering off this, proto-albumose remained in solution.

The liquid from which the precipitate produced by magnesium sulphate had been removed by filtration, contained no proteid coagulable by heat (i.e. no albumin), no proteid precipitable by nitric acid, glacial acetic, or glacial phosphoric acid (i.e. no albumose), but gave a precipitate with tannin, and a well marked xanthoproteic reaction (true peptone). The biuret reaction could not be tried because of the presence of magnesium sulphate.

[1] Up to the time that I received the second quantity of fluid, I had left the specimen of the first which had been saturated with ammonium sulphate unfiltered, so that I was able to compare the amount of precipitate in the two fluids with one another.

[2] The fluid was sent to me by Dr Penrose.

[3] My notes state the fact that the day when this experiment was performed was a hot summer's day, but do not give the precise temperature of the air.

Digestive ferment. In order to investigate the question whether any digestive ferment likely to produce albumose and peptone was present in the liquid, the method I adopted was a very simple one. The liquid was simply kept at 30°—35° C. for several hours, in order that if any proteolytic ferment were present it might exert its action on the proteids in the liquid itself, and so lead to a more abundant production of peptone. I did not investigate what occurred if the liquid were made acid with hydrochloric acid, as the liquid itself is alkaline, and if the peptone and albumose were produced in it by a digestive agent, it must be by one which works in an alkaline medium. The details of the experiment were as follows :—

1. 25 c.c. of the original liquid were saturated with ammonium sulphate thoroughly, and filtered.

2. 25 c.c. of the original liquid were placed in the water bath at 35° C. for four hours, then thoroughly saturated with ammonium sulphate and filtered.

3. 5 c.c. of both filtrates were then taken; to each were added three drops of nitric acid, with which it was boiled, and then three drops of ammonia. The intensity of the yellow colour in the two was the same.

4. 5 c.c. of the filtrates were again taken; to each were added six drops of a solution of tannin; the cloud produced was again equal in the two cases.

There was thus no evidence of an increased quantity of peptone, and thus no evidence of an active proteolytic ferment.

Conclusions. The proteids present were globulin, proto-albumose and peptone. Albumin and fibrinogen were absent. There was no evidence of any proteolytic ferment being present. A small quantity of a substance which reduced Fehling's solution was also present.

CASE 11. Case of spina bifida[1]. First tapping.

The fluid was perfectly clear, colourless and faintly alkaline. A limited quantity of this fluid (about 25 c c.) was obtained, and it was examined as follows :—

It was divided into two parts, one of which was immediately saturated with ammonium sulphate, the other was kept at 40° C. for three hours in a warm bath, and then saturated with ammonium sulphate.

The precipitate so produced was filtered off in each case, and both filtrates were proteid-free: that is there was no peptone in the original fluid, and none had been formed by any process of self-digestion. The precipitate from both filters was washed with saturated solution of ammonium sulphate, redissolved by the addition of water: this solution was heated and a heat coagulum formed at 75° C.; on the removal of which no proteid was found remaining in solution. Another portion of the solution was saturated with magnesium

[1] This fluid was sent to me by Mr A. E. Barker.

sulphate, and the precipitate so produced was filtered off and the filtrate was proteid-free. The precipitate itself was washed and redissolved in the usual way: the solution was faintly opalescent and gave a heat coagulum at 75° C. on the removal of which no proteid remained in solution. The precipitate produced at 75° C. was true coagulated proteid, as it did not dissolve in weak hydrochloric acid or dilute soda, as the precipitate produced by heating hetero-albumose does. Another portion gave an abundant precipitate with nitric acid which did not clear up at all on heating; it lessened somewhat after prolonged boiling, but did not increase again on subsequently cooling.

Conclusions. The only proteid present was a globulin apparently identical with serum globulin. Peptone and albumoses were absent in the original fluid, and as none were formed by a process of self-digestion at 40° C. there was apparently an absence of any proteolytic ferment.

Fibrinogen and albumin were also absent. The reducing substance was not tested for.

CASE 12. Case of chronic hydrocephalus[1]. There was no fever, and the enlargement of the head began soon after birth; the boy came under treatment at the age of six months, when between eight and nine ounces were withdrawn by aspiration. This was during the month of April 1888; on May 14 a similar quantity of fluid was again removed in a similar way, and on June 8th a third quantity approximately the same in amount was similarly removed. The fluid after each operation was sent on to me immediately, and the following are the results of the examination of them.

Fluid removed by the first operation. This was clear, and colourless, faintly alkaline, and of specific gravity 1006.

When a few cubic centimetres were mixed with a equal amount of very dilute Fehling's solution and boiled, there was no formation of any visible red precipitate. The alcoholic extract however when concentrated contained as we shall see later a small amount of a reducing substance. A reducing substance was therefore present, but in so small a quantity as to escape detection in the unconcentrated fluid.

Proteids. A portion of the fluid was boiled after being acidified with a drop or two of 2 per cent. acetic acid. There was a small precipitate, on the removal of which by filtration the filtrate gave evidence of abundant albumose by the nitric acid reaction; it also gave the biuret reaction.

Another portion was saturated with ammonium sulphate. The precipitate so produced was filtered off, the filtrate was proteid-free. Peptone was therefore absent.

A third portion was kept at 40°C. for four hours, and then saturated with ammonium sulphate, and the precipitate so produced was filtered off; the

[1] Under the care of Mr Parker.

filtrate was proteid-free. That is there had been no formation of peptone by any process of self-digestion, and therefore presumably a proteolytic ferment active in an alkaline medium was absent.

A fourth portion was saturated with magnesium sulphate; the precipitate so produced was filtered off, and the filtrate was proteid-free. Albumin and deutero-albumose were therefore absent. The precipitate produced by saturation with magnesium sulphate was washed and dissolved in the usual way. The solution was opalescent, gave a heat coagulum at 75° C. This consisted of true coagulated proteid, not precipitated hetero-albumose, as it was insoluble in weak acids and alkalis. Filtering this off, the filtrate remained clear on boiling, gave a precipitate with nitric acid in the cold, which disappeared on heating, and reappeared on cooling. The original solution of the precipitate produced by saturation with magnesium sulphate was then treated as follows: a few drops of nitric acid were added to it, and a precipitate was produced; this disappeared on heating but not entirely, and became thick again on cooling. There was thus albumose in the original solution; that is to say it had not been produced by the heating of the globulin to a high temperature. The precipitate did not completely disappear on heating because of the presence of globulin.

50 c.c. of the original fluid were then mixed with 300 c.c. of absolute alcohol; the precipitate so produced was allowed to settle, and then the mixture was boiled; the precipitate so obtained was collected on a weighed filter, washed with alcohol and ether and dried to constant weight at 110° C. The weight of the precipitate was 0·0225 gramme; that is the percentage of proteids was 0·045. The alcoholic extract was saved.

Another 50 c.c. was similarly mixed with alcohol; the resulting precipitate was kept under the alcohol for 14 days. The precipitate was collected, and dried at 40° C.; water was then added to the residue which almost entirely dissolved. The insoluble residue (coagulated globulin) was filtered off; the filtrate was clear, and gave no precipitate on boiling after acidification: it gave a precipitate with nitric acid which disappeared on heating and reappeared on cooling; it gave a precipitate with copper sulphate; it gave the biuret reaction: it was not precipitated by dialysing all salt out from the solution. This is another method of showing the presence of proto-albumose.

Some of the watery extract of the alcoholic precipitate was added to dilute sodium sulphate sheep-plasma; but there was no hastening of the coagulation. Fibrin ferment was thus proved to be absent.

The alcoholic extract from this experiment was saved and mixed with that obtained from the previous experiment. The alcohol was evaporated off at 40°, and the residue dissolved in water; the aqueous solution contained a substance which reduced Fehling's solution.

CASE 12. *Fluid removed by second operation.* The fluid was clear, colourless, slightly alkaline, its specific gravity was 1010.

Mixed with an equal quantity of dilute Fehling's solution, and boiled there was a slight amount of red precipitate. The fluid was examined for proteids by precisely the same methods as in the first case; and the results may be thus summarized—

(1) Boiling *plus* acetic acid. Small amount of flocculent precipitate; filtered off. Filtrate gives nitric acid test for albumose, also the biuret reaction.

(2) Nitric acid alone gives a heavy precipitate, which becomes lessened on heating, and thick again on cooling.

(3) Saturation with ammonium sulphate causes a precipitate; filtrate contains no proteid, i.e. peptone is absent.

(4) Saturation with ammonium sulphate, after exposure to 40° C. for three hours. Filtrate contains no proteid, i.e. a proteolytic ferment is absent.

(5) Saturation with magnesium sulphate causes a precipitate (consisting of globulin—heat coagulation 75° C.—and proto-albumose); the filtrate gives a trace of a yellow tinge with nitric acid and ammonia but is practically proteid-free, i.e. albumin is practically absent.

(6) Mixed with serum; no formation of fibrin.

(7) Mixed with dilute salted blood plasma. There was no hastening of the formation of fibrin; i.e. fibrin ferment was absent.

(8) 50 c.c. precipitated with alcohol: weight of precipitate 0·0345 grammes, i.e. 0·069 per cent., which is rather more than in the first tapping.

(9) 50 c.c. precipitated with alcohol; kept under alcohol a fortnight; the proto-albumose was then soluble in water; the globulin (a mere trace) insoluble.

(10) The alcoholic extracts from (8) and (9) were mixed together, evaporated to dryness at 40° C. and the residue taken up with water. The aqueous solution reduced Fehling's solution.

CASE 12. *Fluid removed by third operation.* The fluid was clear, colourless, slightly alkaline. Its specific gravity was 1010.

Mixed with an equal quantity of dilute Fehling's solution there was a well-marked red precipitate. The original fluid was examined for proteids by the same method as in the two previous cases.

(1) Boiling + acetic acid; heavy flocculent precipitate of coagulated proteid; filtered off; filtrate gives faint indication of presence of albumose.

(2) Nitric acid alone gives a heavy precipitate, which on heating did not markedly become lessened.

(3) Saturation with ammonium sulphate causes a precipitate; filtrate contains no proteid, i.e. peptone is absent.

(4) Saturation with magnesium sulphate causes a fairly abundant precipitate consisting of globulin coagulating at 75° C.: the presence of an albumose in this precipitate was doubtful: the filtrate from the magnesium sulphate precipitate contains an albumin coagulating at 73° C.: no albumose.

(5) 50 c.c. treated with excess of alcohol. Percentage of proteids 0·272.

(6) Another 50 c.c. similarly treated; the precipitate was allowed to stand under alcohol for 17 days: it was then nearly insoluble in water: the watery extract did however contain a small amount of proto-albumose.

(7) The alcoholic extract from (5) and (6) was saved; the alcohol evaporated off at 40° C.: and the residue taken up with water. This aqueous solution reduced Fehling's solution. The aqueous extract of the precipitate produced by alcohol did not reduce Fehling's solution. The reducing substance was thus wholly soluble in the alcohol used.

Conclusions. The general conclusions concerning this fluid can be very well put into tabular form thus:—

Fluid from	Specific gravity.	Percentage of proteids.	Varieties of proteids.	Amount of reducing substance (roughly)
1st aspiration	1006	0·045	Globulin (a trace) Proto-albumose	Traces
2nd "	1010	0·069	ditto	More abundant
3rd "	1010	0·272	Globulin Albumin Proto-albumose (a trace)	More abundant still

In the first two cases the result was the same as in the other fluids previously examined. But at the third operation the proteids had become increased in amount; and in kind resembled those present in an ordinary transudation, with the exception that fibrinogen was absent. It would seem from the examination of the fluid that a certain amount of irritation[1] had been set up by two previous aspirations, and thus had caused an effusion to take place from the blood vessels: the percentage of proteids was however not merely so great as in cases of acute inflammation, such as we have seen in Case 7. The presence of a large amount of proteid coagulable by heat rendered difficult the detection of an albumose. It was not until the globulin and albumin had been

[1] Mr Parker tells me that there were no appreciable signs of inflammation, but that the child was after the third operation removed from the hospital and lost sight of.

rendered insoluble by the action of alcohol, that one was able to state positively that an albumose was present.

Further examination of the reducing substance present in Case 12. The alcoholic extracts obtained from the experiments on the proteids were all evaporated to dryness at 40° C. The remainder of the fluid also which had not been used for experiments on the proteids was mixed with excess of alcohol; and the alcoholic extract from this was also evaporated to dryness at 40° C. Altogether in this way I obtained from the three fluids an alcoholic extract of nearly half a litre of cerebro-spinal fluid: it was however only the fluid removed from the third operation that contained an appreciable quantity of the reducing substance. During the evaporation of the alcohol, a light brownish flocculent precipitate separated out, this was filtered off, and was not further investigated; the solid residue was mostly crystalline; it contained abundance of sodium chloride crystals, and a certain small quantity of the feathery crystals like those similarly obtained in Case 7. The residue was taken up with water, and very little remained undissolved. The aqueous extracts from all three specimens reduced Fehling's solution, and did not rotate the plane of polarised light; they were then mixed together, and neutral acetate of lead added: an abundant precipitate was thus produced and filtered off. A stream of sulphuretted hydrogen was passed through the filtrate, and the lead sulphide filtered off; the filtrate was concentrated, and boiled with Fehling's solution; no reduction took place. The reducing substance had thus been entirely precipitated by lead acetate. This precipitate was washed with distilled water, suspended in distilled water, and a stream of sulphuretted hydrogen passed through it till no further precipitate of lead sulphide was produced: the black sulphide was removed by filtration; the filtrate was extracted with ether; the ethereal extract was evaporated to a small bulk, and finally to dryness at 40° C. A small quantity was evaporated to dryness on a glass slide, and examined microscopically. Some slender needles which were colourless and doubly refracting were obtained, together with a small amount of an apparently amorphous substance. The needles were mostly single, but a few groups also occurred. The appearance is depicted in the accompanying wood-cut fig. 1. Fig. 2 represents the appearance presented on evaporating to dryness an ethereal solution of pyrocatechin: the crystals are larger but the shape is the same; these also doubly refract light.

FIG. 1.

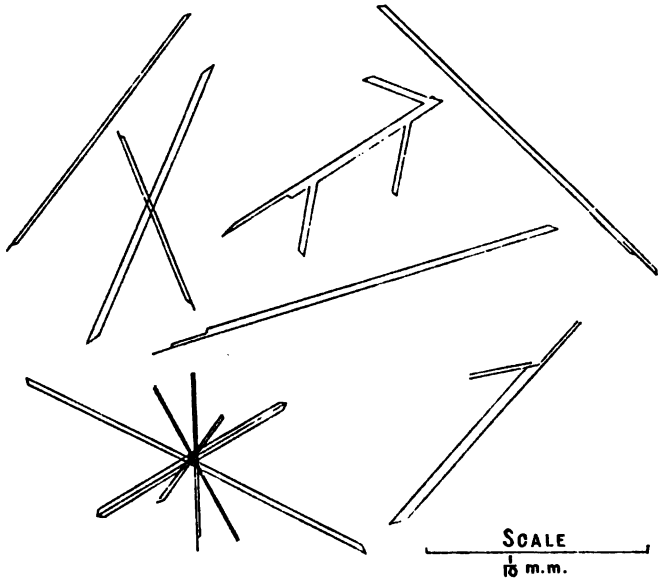
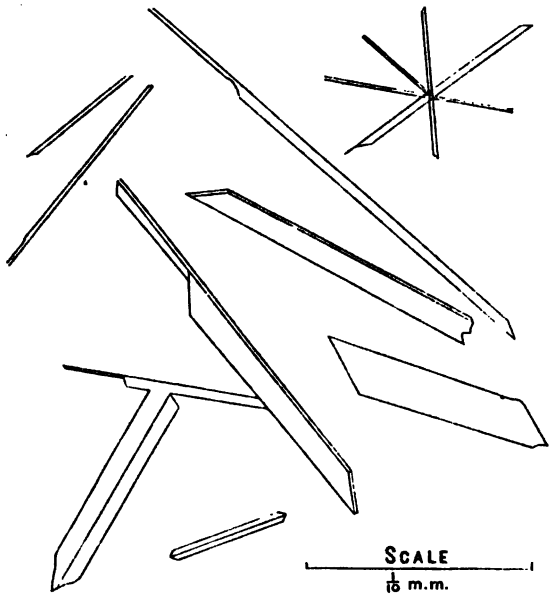


FIG. 2.



About half a decigramme of the crystalline deposit was obtained in all, a quantity much too small for elementary analysis; the following were however its chief properties.

(*a*) At the ordinary temperature of the air, the crystals deliquesced and the deposit became syrupy in consistency.

(*b*) They had an acid reaction.

(*c*) They had a peculiarly pungent bitter taste.

(*d*) As shown by the method of preparation they were soluble in water, alcohol, and ether.

(*e*) Their aqueous and alcoholic solutions did not rotate the plane of polarised light. (Their ethereal solution was not examined with the polarimeter.)

(*f*) They reduced alkaline solutions of copper sulphate on the application of heat; not only was there a red precipitate, but the supernatant fluid became greenish.

(*g*) They did not reduce bismuth salts: this test was performed as follows:—5 grammes of basic nitrate of bismuth, and 5 grammes of tartaric acid were mixed with 30 c.c. of distilled water, and caustic soda added till a clear solution was obtained. A small quantity of this solution was boiled with the aqueous solution of the reducing substance. There was a small amount of white precipitate formed, but no black, or brown colouration, or precipitate.

(*h*) A few drops of 20°/₀ caustic potash were added to some of the aqueous solution of the reducing substance, and after a few minutes' exposure to the air the mixture became yellowish.

(*i*) On the addition of ferric chloride to the solid residue it was turned green.

In all these points the reducing substance exactly resembles pyrocatechin ($C_6H_6O_2$, called also catechol, and oxyphenic acid). Even although I cannot give an elementary analysis, the tests are so numerous that I consider it certain that the two substances are identical: in crystalline form, in solubilities, in reaction, and in taste (which is very striking and peculiar) the two are alike. Both have no action on polarised light; both reduce Fehling's solution with the formation of a green fluid floating above the red precipitate; neither reduce bismuth salts, but examined in the way just described both give a white precipitate: both are darkened on exposure to the air in alkaline solution, and both are turned green on the addition of ferric chloride.

CASE 13. Chronic hydrocephalus in a child aged 18 months[1]. The fluid was removed at the autopsy which took place five hours after death, and it was examined by me immediately.

It was clear, colourless, faintly alkaline and of specific gravity 1008. It contained a trace of a substance which reduced Fehling's solution.

Proteids. The amount of proteid present was exceedingly small, saturation with ammonium sulphate only produced a slight cloud. On account of this only a few observations were made on the proteids; which may be briefly summarized as follows:—

(*a*) Saturation with ammonium sulphate; the filtrate gave a faint tinge of yellow with the xanthoproteic reaction, and a small precipitate with tannin. Biuret reaction *nil*. There was thus a small quantity of true peptone present, too small however to give the biuret reaction.

(*b*) After exposure for six hours to a temperature of 40° C. the fluid was then saturated with ammonium sulphate and filtered; the filtrate gave precisely the same tint with the xanthoproteic reaction; also a similarly small precipitate with tannin. There had therefore been no increase in the formation of peptone due to any process of self-digestion. A proteolytic ferment active in an alkaline solution was therefore absent.

(*c*) Saturation with magnesium sulphate produced a precipitate which was found to consist of globulin coagulating at 75° C., also proto-albumose. The filtrate contained a proteid, as was shown by a faint yellow colour produced by the xanthoproteic reaction, and a small precipitate with tannin. There was however no coagulation on boiling after acidifying with weak acetic acid. The proteid present in the filtrate was therefore the peptone found also in the filtrate after saturation with ammonium sulphate: the tint by the xanthoproteic reaction was approximately the same, as described in (*a*) and (*b*).

(*d*) Nitric acid added to the original fluid gave a precipitate, which almost entirely disappeared on heating, and reappeared on cooling. The albumose was therefore present in the original fluid, and not formed from other proteids by the action of heat. The reason that the precipitate with nitric acid did not entirely dissolve on the application of heat was presumably the coexistence in the fluid of a small amount of globulin.

(*e*) The amount of proteid in the fluid was too small to give any pink or violet colouration with copper sulphate and caustic potash. This reaction is not by any means a delicate test for proteids.

Conclusions. The fluid contained a trace of reducing substance, and a much smaller percentage than usual of proteids; the proteids

[1] I have to thank Mr Murray, Dr Ringer's house physician, for this fluid.

present were globulin, proto-albumose, and small quantities of true peptone. Finding the organic constituents so scanty, I determined to devote the remainder of the fluid I had to the investigation of the inorganic constituents; and especially to the determination of the relative amounts of sodium and potassium salts. C. Schmidt has stated that the percentage of potassium salts is much higher in cerebro-spinal fluid than in other fluids of the body, and it seems to be on this ground chiefly that he speaks of the fluid as a secretion rather than a transudation. Subsequent observers have not confirmed Schmidt's statement, and in this particular case I found that the amount of potassium salts reckoned as chloride formed about four per cent. of the total inorganic constituents, which is about the normal relation throughout the fluids of the body.

The method of analysis adopted is one which seems to me to be worth recording in detail; the actual analysis was performed under the superintendence of Dr Plimpton (Assistant Professor of Chemistry, University College, London); for whose help and suggestions I am greatly indebted. Incineration is a fallacious method, since during ignition certain salts, especially sodium salts, are volatilised; and if Schmidt worked with small quantities of fluid this would perhaps explain his results. In the present case 300 c.c. of the fluid were evaporated to dryness over the water bath, in a porcelain basin; the solid residue was evaporated on the water bath to dryness two or three times with fuming nitric acid to destroy the organic matter, and then two or three times with strong hydrochloric acid to convert the salts present into chlorides; baryta water was then added, until the liquid was just alkaline, and thus lime, magnesia, and phosphates were precipitated, and filtered off. The precipitate was washed with hot water; the filtrate and washings were then concentrated on the water bath, a drop of ammonium carbonate solution being added from time to time to precipitate the excess of baryta. This was filtered off, and the filtrate collected in a platinum capsule, in which it was evaporated to dryness on the water bath, and then ignited carefully at a low red heat to destroy a trace of organic matter which still remained. The residue was dissolved in water, and the aqueous solution evaporated in a weighed platinum dish; the residue = sodium chloride + potassium chloride weighed 2·7825 grammes. This was dissolved in water; platinic chloride was added in excess and thus the potassio-platinic chloride was precipitated; the sodium salt was washed out first with platinic chloride solution, then with alcohol and ether, and the remaining K_2PtCl_6 weighed. The amount of KCl

present was thus calculated to be 0·0859 grammes. In 100 parts therefore NaCl : KCl = 96·15 : 4·85.

General Conclusions and Remarks.

In drawing up the general results of the preceding investigations it will be also convenient to allude to previous researches on the same subject.

General properties and constituents. All previous observers have described cerebro-spinal fluid as a clear, generally colourless fluid, of a faintly alkaline reaction, and of low specific gravity (1006—8). The percentage amount of the chief constituents has been ascertained by Berzelius[1], Mulder[1], Marcet[1], C. Schmidt[2], Méhu[3], Hoppe-Seyler[4], Yvon[5], Müller[6] and Lassaigne[7].

All however show approximately the same results; I shall therefore merely subjoin Hoppe-Seyler's analyses for the purpose of comparison with my own.

Analysis of cerebro-spinal fluid obtained by puncture in two cases of spina bifida.

	I.			II.	
	1st puncture	2nd puncture	3rd puncture	1st puncture	2nd puncture
Water	987·49	986·88	986·72	989·33	989·89
Solids	12·51	13·12	13·28	10·67	10·20
Proteids	1·62	2·64	2·46	·25	·55
Extractives	10·52	2·83	2·65	2·30	2·00
Salts		8·67	8·49	8·02	7·65

These figures sufficiently illustrate the fact confirmed in my own analyses, that cerebro-spinal fluid is poor in the amount of proteid material it contains: and that the amount of salts is approximately the same as in blood and lymph. Schmidt's analyses of hydrocephalus

[1] Quoted by Yvon, see reference below.
[2] Analyses quoted in Hoppe-Seyler's *Physiologische Chemie.*
[3] Méhu, *Chimie médicale.*
[4] Hoppe-Seyler, *Physiol. Chemie*, p. 601.
[5] Yvon, *Journal de pharmacie et de chimie*, Série IV. Vol. XXVI. 1887, p. 210.
[6] Fr. Müller, *Mittheil. a. d. Würzburger med. Klinik*, Vol. I. p. 267.
[7] Quoted by Thiéry, *Progrès medicale*, Vol. XIV., p. 286 (1885).

fluid show a rather higher percentage of proteids than Hoppe-Seyler's analyses of spina bifida fluid.

Most interest however is attached to the individual constituents themselves, apart from their quantity, and I shall therefore take up separately the proteids, reducing substance, and salts.

Proteids of cerebro-spinal fluid. Yvon investigated one specimen of hydrocephalus fluid, and he found in it proteids which he named hydropisine, métalbumine, and serine; the nomenclature indicates that the methods adopted for the separation of proteids were somewhat old-fashioned, and thus the observation is of but little value. Fr. Müller in a case of hydrocephalus fluid found both globulin and albumin: but no exact details are given of the method adopted for separation. Most of the other observers mentioned merely give the quantity of total proteid present, or more generally use the word albumin synonymously with proteid.

Hoppe-Seyler[1] however recognised that there is something peculiar about the proteids present: he says that boiling gives a cloudiness only, because the quantity of proteid is small, and the liquid is alkaline. There is nothing peculiar about that; a weak, alkaline solution of any albumin or globulin does not give a flocculent precipitate on heating until it is rendered faintly acid. Hoppe-Seyler then goes on to say that although he had at one time considered that the proteid present was of the nature of casein, later investigation had led him to believe that it was a globulin. He further states that fibrinogen is absent; there is no coagulum on heating to 56°C. and no formation of fibrin on admixture with serum. This is so far as I can find the only trustworthy statement previously made with regard to the nature of the proteids.

Let me now give a résumé of the investigations, so far as they relate to the proteids, which I have made.

[1] *loc. cit.* p. 607.

Case.	Quantity of Proteid.	Kind of Proteids present.
1. S. B.[1]	0·0842 per cent.	Globulin; albumose of doubtful variety
2. S. B.	0·1602 „	Not investigated
3. S. B.	0·0199 „	Globulin
4. S. B.		Globulin; proto-albumose; deutero-albumose
5.[2]		Albumose present; other reactions doubtful
7. H.	0·65	Fibrinogen; serum globulin; serum albumin; proto-albumose (acute case)
8.[3]		Globulin; proto-albumose
9. S. B.		
1st tapping		Globulin; albumin; proto-albumose
2nd „	Proteids more abundant than in first tapping	Globulin; albumin; proto albumose
10. H.		Globulin; proto-albumose; peptone
11. S. B.		Globulin
12. H.		
1st aspiration	0·045	Globulin (traces); proto-albumose
2nd „	0·069	Globulin; proto-albumose
3rd „	0·272	Globulin; albumin; proto-albumose (traces)
13. H.		Globulin; proto-albumose; peptone (traces)

In two cases the proteid present consisted wholly of globulin. In six cases, the proteids consisted of globulin and albumose. The albumose present was generally proto-albumose: in one case deutero-albumose was present also; in two cases peptone was present in addition to albumoses, that is true peptone in Kühne's sense; a proteid which is not precipitable by saturation with ammonium sulphate. In three cases albumin was present, but two of the three cases may be excluded from the general classification; Case 7 was a case of acute inflammation, and in Case 12 (third tapping) there was also doubtless a chronic or subacute inflammatory condition; that leaves us with Case 9 as the only instance in which apparently normal cerebro-spinal fluid contained albumin.

Fibrinogen was absent throughout (except in Case 7, which as before mentioned was not an instance of the normal fluid but is useful for the sake of contrast with the normal fluid). Addition of cerebro-

[1] S. B. means spina bifida. H. means hydrocephalus.
[2] Case of myxoedema. [3] Case of cerebral meningocoele.

spinal fluid to serum never caused the formation of fibrin; heating the cerebro-spinal fluid to 56° C. also caused no precipitate. The globulin present is one which like serum globulin is coagulated at a temperature of 75°. It is not cell globulin[1], as when tested with dilute magnesium sulphate plasma it has none of the properties of fibrin-ferment.

The interest however of the proteids seems to me to be the presence of albumoses, and peptones: on investigating the question as to whether the fluid contains any digestive ferment to produce these, the experiments I have performed, and which have already been described (pp. 242-3), gave in all cases a negative result.

Peptone has from time to time been described as a normal constituent of blood, milk, pus, and other fluids and tissues of the body. As Neumeister[2] has recently pointed out such observations were made previous to the discovery of the use of ammonium sulphate as a means of separating peptones from other proteids, and are therefore untrustworthy. He also considers it possible that in the heat coagulation of albumins and globulins, a small amount of primary albumoses (i.e. proto- and hetero-albumose) may be formed by the hydrating action of the acidified hot water. This action must however be very small when the acidification is so slight, and the heating is prolonged for so short a time (two or three minutes) as it was in my experiments. I however in several cases obtained evidence of albumoses in the original fluid, and in Case 12 separated the albumoses from other proteids by the use of alcohol; alcohol precipitates all the varieties of proteid present, and its prolonged action coagulates (i.e. renders subsequently insoluble in water) the globulin, and leaves the albumoses still freely soluble.

I tested the question as to the presence of albumoses in other fluids, by first filtering off the proteids coagulable by acidulation and boiling, and then testing for albumoses by the nitric acid and biuret reactions. In this way I examined seven specimens of blood (man, horse, sheep and rabbit), two specimens of pericardial fluid (horse), one specimen of pleuritic fluid, four of ascitic fluid, four of fluid removed from blisters, and three of hydrocele fluid. As a rule the filtrate gave a faint xanthoproteic reaction; in a few cases there was not sufficient proteid to give even that; but in all cases albumoses were absent.

Reducing substance. The existence of a reducing substance in

[1] See my paper on the Nature of Fibrin Ferment: this *Journal*, Vol. IX. p. 229.

[2] Neumeister, *Zeitschr. für Biologie*, Vol. XXIV. p. 272.

cerebro-spinal fluid has long been known. Claude Bernard[1] spoke of it as sugar. Bussy and Deschamps[2] found a reducing substance in the normal cerebro-spinal fluid of the horse and dog, as well as in a specimen obtained from the ear of a man who had sustained a fracture of the base of the cranium. In none of their experiments however were they able to induce the alcoholic fermentation. The idea that the substance in question was not sugar but something else, did not strike them, and it was Turner[3] (who examined the fluid removed from a case of spina bifida by Sir James Paget) who made the suggestion that probably it was not a carbohydrate at all, but some derivative of albumin that possessed the reducing action.

Since that time, the existence of a reducing substance in cerebro-spinal fluid has been a matter of common observation. In addition to not undergoing the alcoholic fermentation, the fact has also been observed that it has no action on the plane of polarised light. This negative evidence I have been able to confirm, and to add to it the additional point that it does not give the phenyl-hydrazine test for sugar[4].

Gorup-Besanez[5] suggested that the substance was the same as Bodeker's alcapton[6], a statement to which my attention was first drawn by a paper by G. Thiery[7]; but I am not aware that this supposition has ever before been put to the test of experiment. Alcapton was the name given by Bodeker to a reducing substance occasionally occurring in urine, and which has since been shown to consist of pyrocatechin or allied aromatic substances. The observations detailed under Cases 7 and 12 (pp. 238 and 247) have convinced me that the substance is pyrocatechin: though whether this substance is present free, or in some combined condition I am unable to state.

Hoppe-Seyler states that the reducing substance (which he speaks of as sugar) is not a normal constituent of cerebro-spinal fluid, but only

[1] Quoted by Gorup-Besanez, *Lehrbuch*. Vol. II. p. 397.

[2] *Bulletin de l'academie de médecine*, Dec. 1852.

[3] *Royal Society, Proceedings*, Vol. VII. (1854), p. 89.

[4] A fact which also excludes glucosamine, a nitrogenous reducing substance (see F. Tiemann, *Berichte d. Berlin chem. Gesellschaft*, Vol. XIX. p. 49). Glycuronic acid is excluded by the fact that the reducing substance in cerebro-spinal fluid is optically inactive, is soluble in cold alcohol, is completely precipitated by neutral lead acetate, and does not reduce bismuth salts.

[5] *loc. cit.*

[6] *Zeitschr. f. rat. Med.*, Vol. VII. p. 128.

[7] Thiery, *Progrès médicale*, Vol. XIV. p. 286.

appears subsequent to the irritation set up by tapping. In my own work I have never failed to find the reducing substance; its quantity however is very much increased by repeated tapping or aspiration. The fluid removed by the first operation may require to be concentrated before it gives the test in question.

Salts. C. Schmidt made several analyses of hydrocephalus fluid and remarked on the high percentage of potassium salts present. I quote three analyses[1].

	Inorganic salts in parts per 1000.		
	I	II	III
K_2SO_4	0·096	0·193	0·222
KCl	2·181	1·485	0·232
NaCl	4·438	4·101	6·054
Na_3PO_4	0·613	0·486	0·115
Na_2O	1·842	2·290	0·987
$Ca_3(PO_4)$ / $Mg_3(PO_4)_2$	0 307	0·362	0·271

In two out of the three cases as is seen, there is certainly a higher percentage of potassium salts than occurs in blood and lymph.

Subsequent investigations have however shown that this is quite exceptional; the following are a few analyses that I have been able to find.

Lassaigne gives the following numbers: in fluid removed from an old woman, the total quantity of salts in parts per 1000 was 8·54, of which 8·01 consisted of sodium chloride; in the cerebro-spinal fluid of the horse he found total salts per 1000, 6·89, of which 6·10 consisted of sodium chloride.

Yvon examined the fluid from a case of hydrocephalus and found total salts per 1000, 8·90, of which 7·098 consisted of sodium chloride.

Fr. Müller's case was also one of hydrocephalus: he found the total salts per 1000 to be 8·80; and the ratio NaCl : KCl = 21·5 : 1.

In the one case (Case 13) in which I have investigated this question, the ratio of the two salts was nearly the same as in Müller's case: viz. NaCl : KCl = 95·15 : 485.

[1] Taken from Hoppe-Seyler's *Physiol. Chemie*, p. 604.

The analysis is fully described on p. 251, and I would specially draw attention to two points in the method adopted: (1) that a large quantity of liquid, 300 c.c. was taken, and thus errors due to manipulation were minimised: (2) organic matter was destroyed, not by ignition but by means of fuming nitric acid: by this means the passing off of volatile salts like sodium chloride was entirely prevented. Ignition was employed in a later stage to destroy the last traces of organic matter; but it is when incineration is performed in the presence of a large quantity of carbonaceous matter that the danger of salts like sodium chloride being carried off is especially to be feared.

As regards the saline constituents, it can therefore be said that there is no peculiarity in cerebro-spinal fluid. It is in the exceptional character of the proteids, and in the presence of pyrocatechin, that this fluid stands clearly marked off from serous or lymph-like fluids. Several interesting questions arise out of an investigation like this, but with which I have not attempted to deal. Such questions are those relating to the secretion of the fluid; where and under what circumstances is it secreted? and is the function of the fluid excretory, or nutritive? or is the composition of the fluid merely accidental, and its function merely the mechanical one of exercising a necessary pressure upon the organs it bathes?

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ON THE ELECTRICAL ORGAN OF THE SKATE. PART II.

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Pl. XXII.

In the previous paper[1] several questions were enumerated as still requiring investigation, particularly the nature of the normal reflex process by which the electric organ is discharged, and the measurement of the electromotive force of the response of the organ to a single excitation. The following observations relate chiefly to these points.

SECTION I. THE REFLEX.

It was stated in the last paper that the skate can be at any time induced to discharge its organ by rubbing the surface of the skin. The response consists of one or more electrical shocks of which the direction is always from the root to the tip of the tail. To observe this effect conveniently the skate is secured with its ventral surface applied to a board of similar shape to that of its own body. The board is plunged in a tub of sea water, which covers the spiracles, leaving the tail and hinder part of the body exposed. It is then easy to apply cables to the tail as described in our first paper and to connect these either with the rheoscopic frog preparation or, by means of non-polarisable electrodes, with a galvanoscope.

It was before stated (p. 145 last paragraph) that we were unable to obtain discharges by electrical stimulation of the skin. This year however we succeeded in doing so, using needle electrodes, which were introduced subcutaneously two or three inches apart. No response could be obtained when single induction shocks were used, unless the intensity was so great as to give rise to unipolar escape, but repeated excitation by faradisation (with or without the side wire) yielded summation results which could be depended upon.

[1] This *Journal*, Vol. IX. pp. 137—166.

The time and character of the response could no doubt be recorded with much greater advantage by photography than by any other method, but the apparatus necessary for the purpose was not at our disposal. We had therefore recourse to the rheoscopic frog preparation, which was so arranged as to inscribe its contractions on a blackened surface of which the rate of movement (4 centim. per second) was measured by recording at the same time the vibrations of the hammer of the induction apparatus, for which purpose an electro-magnetic time-marker was introduced into the circuit of the primary coil.

The axis of the recording cylinder was horizontal, and the bell-crank lever of the myograph worked on a vertical axis. The long arm carried a straw with a parchment pen cemented to its tip. The muscle acted on the short arm, its action being resisted not by a weight, but by an india-rubber spring attached to a thread which was wound round the axle of the lever. The myograph was connected either with the toes of the rheoscopic limb, so as to take advantage of the greater excitability of the peroneal muscles, or with the tendo achillis. The mode of procedure is as follows:—

The cables attached to the tail are laid on a couple of wedge-shaped lumps of kaolin, which rest on a glass plate, and placed at a distance of a centimeter from each other. The block of the myograph to which the rheoscopic preparation is fixed is now brought into such a position that the nerve can be made to bridge in air the two kaolin masses.

In a fresh preparation of R. maculata it was found that the rheoscope responded after seven vibrations of the hammer. As the rate of vibration had been ascertained by comparison with that of a tuning-fork to be 50 per second, this gives a delay of 0·14″, from which probably 0·02″ ought to be subtracted for delay due to muscle latency and to mechanical causes. It will be noticed that the experiment affords no information as to the true reaction time. It shews nothing more than that here as in other cases of electrical stimulation of the skin a reflex response can only be obtained by summation of stimuli.

In all our experiments relative to the reflex process, excepting the one above described, mechanical excitation of the skin was preferred to electrical, as being much more certain.

In the observations made this autumn at Plymouth in which four species of skate were used, we met with no instance in which discharge could not be evoked by acting mechanically on the skin. The species used were *R. clavata*, *R. Batis*, *R. maculata* and *R. microcellata*. In the two

last mentioned, which were not at our disposal at St Andrews, the organ presents the same structural characters as in *Batis* and *clavata* and is quite as active functionally. In animals in a good state, mere pressure with a vulcanite rod on the dorsal surface near its lateral border was effectual; in others, results could only be obtained by rubbing. It need scarcely be observed that in both cases there is summation. Responses can also be obtained by acting similarly on the ventral surface, but with much greater difficulty and uncertainty.

In order to determine with certainty whether or not a response occurs, the rheoscopic frog preparation is not suitable, for contractions are only obtained when the shocks are of considerable intensity. It is therefore necessary for the investigation of the reflex, to use a much more delicate galvanoscope. For this reason all our experiments were so arranged that we could at the same time observe the rheoscopic contractions of the limb and the indications of a capillary electrometer connected with the cables already mentioned by nonpolarisable electrodes. It was then seen that responses which are much too feeble to excite contractions give large excursions of the mercurial column.

With a view to the determination of the afferent channels by which the reflex can be evoked, our attention was first directed to the fifth nerve. In the skate, this nerve furnishes a large branch to the sensory ampullae, the tubes of which spread over a considerable part of the dorsal surface of the animal. It seemed not improbable that they might have some importance as afferent channels.

Before proceeding further, it may not be out of place to refer to the extraordinary mistake made first by M. Jobert de Lamballe (1858) and subsequently (1861) by Dr Macdonnell[1], both of whom regarded the bunch of ampullae and the nerves distributed to them as a single organ and believed it to be the homologue of the electrical organ of Torpedo,—in M. Jobert's words, a rudimentary electric apparatus. This misconception would have had little interest in connection with our present enquiry, had it not unfortunately misled Mr Darwin in his discussion of the difficulties which the electrical organs of fishes offer in this relation to the theory of Natural Selection (*Origin of Spec.* p. 150, 6th Edition). Under this head Mr Darwin remarks 'In the Ray...there is an organ near the head not known to be electrical, but which appears to be the real homologue of the electric battery of the

[1] Jobert (de Lamballe), *Des Appareils Électriques des Poissons Électriques.* Paris 1858, p. 39—43. Dr R. H. Macdonnell, "On an organ in the skate which appears to be the homologue of the electrical organ of the Torpedo." *Nat. Hist. Review* 1861, p. 57.

Torpedo." In the *Life* (Vol. II. p. 353) a letter is printed in which he refers to Dr Macdonnell's discoveries.

It may be asked on what ground M. Jobert concludes that this organ, the true structure of which had been made out by Leydig as early as 1852, is possessed of electrical properties[1]. The answer is, that without making any experiments, he conjectured that since it resembled the organ of Torpedo in anatomical position, in consisting of a tube charged with gelatinous semifluid material, and in being richly supplied with nerves, the two must correspond in function! It is scarcely necessary to add that there is no real resemblance either in structure or function. Experiment shews that the organ is electrically inactive.

The branch of the fifth nerve which ends in the sensory ampullae is at least three times as large as any other branch. It can be easily divided without opening the cranial cavity, and we were able by exciting electrically the central end of the divided nerve to evoke a discharge; but in repeated experiments in which the production of discharges by mechanical excitation of the skin on the same side and on the opposite (uninjured) side, were compared, no difference could be observed, whatever part of the dorsal surface was acted upon.

The next step was to ascertain whether the division of the trunk of the trigeminus put an end to the reflex effect of stimulation. This was easily accomplished by plunging a narrow bladed knife through the lateral wall of the brain case, so as to sever the nerve in its cartilaginous canal. The first experiment shewed distinctly that after division a response could be got by rubbing the skin at least as easily as before.

We next made a sagittal incision in another skate (R. maculata) beginning from the inner wall of the orbit and extending backwards for half an inch. By this incision, as was afterwards ascertained, all the cranial nerves were divided, excepting the olfactory and vagus group. The rubbing reflex could still be obtained. Finally in another skate (R. clavata) the division was extended backwards on the right side so as to include the vagus and forwards to the anterior border of the animal's body. The knife was then passed through from the dorsal to the ventral surface so as to sever all structures to the right of the line of incision from the cranium and snout. The reflex response occurred as easily as

[1] Of this anyone may satisfy himself by comparing Jobert's elaborate Plates with the woodcut in Leydig's work. *Beiträge zur mikr. Anat. der Rochen und Haien*, Leipzig 1852, p. 41.

before the incision. We then immediately made a similar severance on the other side. The reflex result was still obtained and with equal readiness, from mechanical excitation of all parts of the dorsal surface The experiment was repeated in two other animals with the same results. It is clear therefore that the channels of influence are chiefly spinal.

The reflex centre. The following experiment, which was repeated five times with unvarying result, the specimens used being R. maculata or clavata, shews that division of the spinal cord abolishes the reflex. A skate (R. maculata or microcellata) having been prepared in the way already described, it was first ascertained that electrical discharges could be easily produced by rubbing. The cord was then exposed immediately behind the medulla and a ligature passed under it, great care being taken to avoid the entrance of sea water into the spinal canal. The reflex was again tested by the electrometer and found present. On tightening the ligature, a momentary discharge occurred after which no further responses could be obtained reflexly, the centre therefore must be chiefly intracranial.

We then proceeded to localise the centre. The following facts were ascertained:—after removal of the olfactory lobes and hemispheres the reflex still persisted; the organ discharged at least as readily in response to stimulation of the skin as before. No discharge occurred at the moment of severing the lobes. Removal of the optic lobes in the same preparation immediately abolished the reflex. The act of removal was accompanied by a discharge. It was followed by no change of the movements of the spiracles either as regards rhythm or frequency.

The channels of influence between cutaneous end organs and electrical organ are therefore afferent channels which for the most part enter the spinal cord and end in the optic lobes, and efferent channels which, starting from the lobes, pass down the spinal cord to the organ.

The character of the discharge varies according to the mode of mechanical excitation and the state of the preparation. We will first describe what is observed with the aid of the physiological rheoscope.

In a perfectly fresh preparation the discharge, as indicated by the muscular contractions of the rheoscopic limb, has the character of an irregular tetanus (Plate XXII, Figs. 1 and 2) of which the frequency is from 20 to 25 per second. This effect however can only be obtained once or twice. The subsequent muscular responses consist either of single contractions or successive thrills which follow each other at

intervals of half a second or more. Each thrill is made up of two or three twitches. In the intervals between the thrills the muscle remains partly contracted. This is shewn in the Plate, Fig. 3. In some cases the tracings of the thrills resemble single contractions, but when they are compared with those of real single contractions of the same muscle and are examined more carefully, it is seen that they are such as would be produced by two effective stimuli following each other at an interval of $\frac{1}{15}''$ to $\frac{1}{20}''$. In making a series of observations on the same animal, it is observed, that whereas the earlier muscular effects have for the most part the character of thrills, the later ones are chiefly single twitches or couples such as those above described shewing superposition.

In almost all the observations with the rheoscopic limb, the electrometer was observed at the same time, the terminals being connected as already explained with the cables which received the discharge. The connection was such that the passage of the discharge drove the meniscus from the point of the capillary. The electrometer was made for the purpose. It is of quick reaction, but not very sensitive. Its zero pressure is 20 centimeters. It is read by a microscope provided with an eye-piece micrometer scale. A displacement of the column which measures 50 divisions of this scale corresponds to a difference of potential of 0·15 Daniell. When the two methods of observation were used together, it was seen that at first every contraction was accompanied by a sudden projection of the mercury column out of the field. As the effects became weaker and the contractions began to fail, the character of the excursions of the electrometer could be observed, and it was then seen that each discharge was indicated by a leap up of the column through from 60 to 100 divisions, from which point, after remaining for a moment in rapid oscillation, it descended in steps. Comparing this effect with what occurred at the same moment in the rheoscopic muscle, it was obvious that the muscular response was a single twitch coinciding with the leap up of the column.

Long after the response of the rheoscopic muscle has ceased each discharge can be observed by the electrometer, the excursions of which by reason of their diminished amplitude can be studied with much greater advantage than before, and afford indications, which if we had been able to record them photographically, would have been of great value. All that could be done was to observe and describe the phenomena as they presented themselves.

The effect varies according to the way in which it is evoked. When the vulcanite rod is smartly drawn across the skin, the response is that

already described. When it is thrust against the skin there is usually a single leap of the column of such short duration that it cannot be followed by the eye. When it is drawn slowly with pressure the effect is of the same kind as that first described, but more prolonged, and it is then seen that the oscillations are in groups, the first of each group being higher than the rest.

The frequency of the oscillations in a group is sometimes too great to admit of their being counted, but usually it does not exceed 8 per second, declining to 5 or less when the observation is continued. The groups recur at intervals of from half a second to a second.

Electrical Excitation of Central Organs.

The electrical organ can be made to discharge by excitation either of the optic lobes, or of the spinal cord after division behind the medulla oblongata. We will first describe the phenomena observed in the latter case.

Spinal cord. No effect can be produced by single induction shocks. It was shewn in our former paper that when the spinal cord is exposed near the root of the tail, a single induction current is effectual and produces an apparently instantaneous electrical response, which however is found when investigated by the rheotome to be made up of several single effects fused together. But nothing of the kind was observed in excitation of the cervical or thoracic part of the cord.

Excitation by the alternating currents of the induction apparatus is effectual in any part of the cord. For obvious reasons the inductorium was provided with Helmholtz' side wire. As the hammer vibrated 50 times per second, the rate of the alternating induction currents was 100, i.e. 50 in each direction. The electrodes were needles, insulated excepting at their points. They were placed a centimeter apart, the cord having been previously divided behind the bulb, and exposed to a sufficient distance backwards.

As in the previous experiment, the character of the response was observed by simultaneous observation with the rheoscopic limb and the electrometer. When the cord was excited for a second or more with the secondary coil at 50 of the scale, i.e. at such a distance from the primary that the shock could just be felt by the tongue when the electrodes were applied to it, a single contraction of the rheoscopic muscle occurred at the moment that the excitation began, and a second at the moment that it ceased, as shewn in the Plate, Figs. 6 and 7. It is

seen that the first response occurs several hundredths of a second after the beginning of the stimulation, and that when the period is short the second effect is stronger than the first. Even when the period was more than 6 seconds, there was no contraction excepting the initial and terminal one. By the simultaneous observation of the electrometer it is seen that at the beginning of each excitation period the column leaps up, and again at the end. Between the two leaps the meniscus oscillates a little above its zero position, indicating that although the muscle is quiescent during the interval between the initial and terminal contractions, the organ is in a state of excitation all the time. It seems at first sight as if this might be explained on the supposition that the excitatory effects in the organ are of such duration as to be nearly continuous, so that the change of electrical state which each represents is relatively inconsiderable. This explanation however is seen to be inadequate when it is considered that the time-interval between each excitation and its successor is probably not less than a tenth of a second, whereas the duration of the excitatory effect is not more than a fiftieth of a second, so that after each several excitation the organ has more than time to return to its condition of rest; the change is therefore as abrupt from this state to that of excitation the second, third or twentieth time, as the first time. All therefore that can be said in reference to the initial and terminal contractions is that the excitations during the interval are inadequate to excite the rheoscopic nerve, although abundantly sufficient to affect the electrometer. This means nothing more than that the intensity of the excitatory effect is greater at the end and at the beginning than during the interval. Why this is so, it is not at present possible to explain. It may be noted that an analogous muscular effect, "*tétanos à renforcement*," presents itself in electrical stimulation of the mammalian cortex[1] and spinal cord[2].

In all experiments of the kind above described the dorsal muscles of the fins are during the excitation thrown into tetanic contraction. The lateral borders of the fins are arched strongly upwards so that they nearly meet above the body, and are suddenly relaxed when the excitation ceases. At the same time the caudal fins are thrown into spasms, but on comparing the modes of contraction of the two groups of muscles it

[1] See François-Franck, *Leçons sur les fonctions motrices du cerveau*, p. 73.

[2] In some recent experiments at present unpublished made by Horsley and Gotch the same phenomenon has been obtained during excitation of the spinal cord of the cat, as evidenced by the muscular contractions.

is found that whereas those of the back are in complete tetanus, the others thrill rhythmically with a frequency of 6 to 8 per second, as can be easily observed by recording their movements graphically. This evidently means that the dorsal muscles are made to contract by excitation of their motor nerves, those of the tail fins by excitation of the spinal cord, and accordingly the latter have a rhythm identical with the similarly excited discharges of the organ.

The fact that the organ responds rhythmically to excitation of the spinal cord indicates that the cord acts as a centre. This is put beyond question by the observation that in all cases in which the divided cord is excited for a sufficient time, not less than a couple of seconds, there occur after excitation has ceased, electrical discharges following each other at short intervals. This can only be explained as meaning that, as a consequence of excitation, the spinal centres discharge themselves automatically, just as in the higher animals epileptiform disturbances spring from similar automatic action in parts of the cortex which have been excited.

The nature of the effect can be best judged of by the following details:—

(1) In a skate (R. microcellata) 32 inches long and 18 inches across the fins, the spinal cord was exposed and divided behind the medulla oblongata. The cord was excited for 2 seconds by alternating induction currents (100 per second); the column of the electrometer which was at zero when the organ was unexcited, shot up to 60 scale at the beginning of this period, oscillated at about the same height, and again shot up to 90 scale at the moment that the excitation ceased, and then suddenly fell to its previous position. After remaining at zero for 3 seconds, it leapt to 70 scale, returning instantly. After another pause of the same duration, it rose in steps to 60 scale, oscillated for a moment and fell, the whole excursion lasting two seconds. A third pause of 6 seconds was followed by a similar excursion in which the column did not rise above 20 scale, after which it remained quiescent.

(2) A skate (R. maculata) 23 inches long and 15 wide was observed in the same way. Excitation for 2 seconds, during which the indications of the electrometer were those described above. Two seconds after the excitation had ceased, a single excursion was observed, i.e. one in which the column flashed up for a moment and returned to zero. There was then a pause of about a second which was followed by three such single excursions in quick succession. After another pause of the same duration, the column shot up and oscillated ten or twelve times

within a period of 2 seconds and then fell. A fourth similar pause was followed by two single excursions in quick succession, and finally, after a moment's rest a third. As in this observation all the pauses, excepting the first, lasted about a second—less or more—the automatic discharges continued to recur for a period of about 10 seconds.

(3) In another specimen (R. microcellata 24″ × 15″) the column rose in steps during the whole period of excitation, shooting up at the end, but instead of at once falling to zero as in the previous examples, subsided slowly, oscillating the whole time, its decline being interrupted at intervals of about a second by leaps in which it rose nearly as high as it had done at the end of the period of excitation. There was no sensible difference between the oscillations which occurred during excitation and those which followed it. The frequency throughout was 6 per second.

It will be understood that when the period of excitation is too short, the organ responds during excitation, but there are no automatic discharges after it. The duration required differs in different animals. Excitations of 2 seconds duration were followed by automatic discharges 17 times out of 27. After those of 5 seconds, these were observed 7 times out of 8. It constantly happened that when the short excitation had ceased to produce the effect, it recurred when the time was prolonged. The automatic discharges were in most cases not accompanied by visible contractions in any of the muscles of the fish.

Optic lobes. For exciting the organs within the cranium, electrodes, of insulated platinum wire, of which the exposed tips were 1 millimeter apart, were substituted for the needles. In other respects the method was the same.

No discharge could be produced by exciting any part of the hemisphere or the cerebellum, but the moment that the electrodes were brought into contact with the surface of the optic lobes, the rheoscopic limb contracted, and the mercurial column shot out of the field. Here, as in the spinal cord, a single induction shock is without effect and in order to produce a discharge the duration of the interrupted electrical excitation had to be more than a second. The rheoscopic muscular effect, during the period of excitation may either be made up of (*a*) groups of single twitches, or (*b*) an initial contraction or couple of contractions followed after a longer pause of say half a second, by another couple or group of exactly the same kind; or (*c*) it may consist of a single initial contraction only. The contractions of a group follow each other at intervals of about $\frac{1}{8}$ second as shewn in the

Plate, Figs. 4 and 5. When the electrometer was observed at the same time with the rheoscopic muscle, the mercurial column was seen to be, as in the case of excitation of the spinal cord, in a state of oscillation (6 or 8 per second) during the whole period of excitation. At the beginning of each period the column ascended by rapid jerks towards a summit, then oscillated for a moment at a lower level and again culminated, repeating this process at intervals of half a second or less: each summit corresponded to a group of contractions of the muscle, which during the intermediate period of oscillation was inactive. Here again the electrometer effect could be observed long after the rheoscopic preparation had ceased to respond, and from first to last had the same character, the only difference between the later and earlier observations being that the frequency of oscillation diminished as time went on, from 8 to say 5 per second. It is well to notice that although in several instances we failed, even at the beginning of the experiment, to obtain responses from the rheoscopic frog, the discharges could always be observed by the electrometer.

As in the experiments on the spinal cord, so in those now to be described, excitation of the lobes was followed by a period during which automatic discharges of the excited centre occurred at intervals. These were not sufficiently strong to evoke rheoscopic contractions, but could be observed by the electrometer.

(1) In a specimen of R. maculata, 26″ × 19″, excitation of the optic lobes for 2 seconds, which produced the usual concomitant effects, was followed after an interval of five seconds by a series of 14 discharges. Each was indicated by a sudden leap up of the column, which oscillated at the height first attained for a fraction of a second and then fell. Between each of the first four and its successor there was an interval of about a second, the intervals subsequently lengthening first to 4 seconds and finally to 9 seconds, so that the whole effect lasted for nearly a minute.

(2) In another fish (R. clavata, 30 inches long and 24 wide) excited for two seconds, the mercurial column instead of falling, continued to oscillate at the same height for 5 seconds after the excitation had ceased. It then fell to a lower level and continued to oscillate for 10 seconds more, gradually subsiding. No further effect was observed.

As each of these varieties of effect was observed repeatedly in different fish, and as both occurred several times in the same specimen, it is not possible to regard either as abnormal. The reader will see that between these effects and those which follow excitation of the spinal

cord, there is only this difference, that the former last longer and are of greater intensity. In other respects they are analogous. Both optic lobes and cord are capable of automatic action, but our observations lead us to believe that the optic lobes alone contain the centre through which the organ can be excited reflexly.

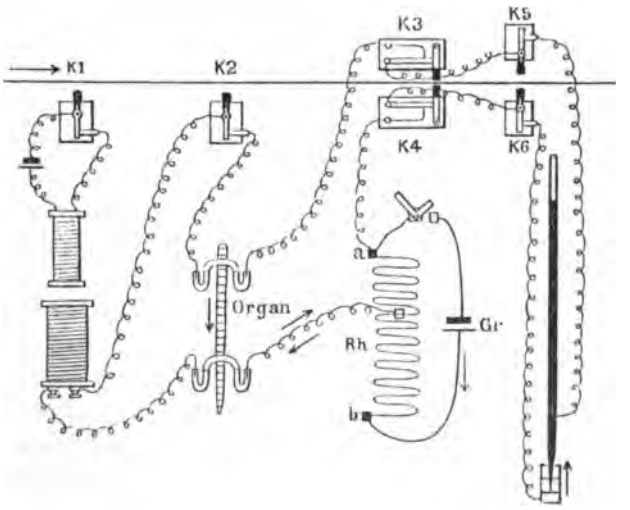
In exposing the brain, sea water sometimes entered the cranial cavity in spite of the care we used to prevent it. Whenever this happened, it declared itself by the occurrence of vigorous discharges of the organ, which were accompanied by convulsive movements of the body. The discharges lasted for many minutes and were at first violent. When they had ceased no further response could be evoked by excitation, either of the optic lobes or cord. Thus in a specimen of R. maculata (21 inches long by 16 inches wide) in which this accident happened and in which contemporaneous observations were made of the rheoscopic preparation and of the electrometer, the early discharges were accompanied by tetanic spasms of the rheoscopic muscle, each spasm being made up of single but unequal contractions, which succeeded each other with a frequency of from 10 to over 20 per second. Three of these effects are shewn in the Plate, Figs. 8, 9 and 10. Subsequently, as they gradually diminished, each discharge was represented by a single initial contraction, and after these rheoscopic contractions had ceased, the electrometer effects could be observed for five minutes. The earlier effects, i.e. those which were strong enough to excite the rheoscopic preparation, were accompanied by excursions which were of such extent that it was necessary to bridge the kaolin electrodes of the electrometer by a moist thread. In each excursion, the column first leapt to the top of the scale, oscillated at the height it had attained for a fraction of a second and then fell. The subsequent excursions were similar but of less amplitude.

In another instance (R. maculata, 20 inches long by 12 wide) we had reason to believe that sea water had entered the cranium, although at first no discharge occurred. On stimulating with alternating induction currents of the usual strength, there was no response, but on doubling the strength, a discharge of the usual character occurred during excitation, which was followed by a series of automatic discharges at intervals of from 1 to 5 seconds, which continued for a period of 7 minutes. In this case the results were observed by the electrometer only, the indications of which correspond exactly with those last described. At the end of the period no responses could be evoked by stimulation either of the lobes or cord.

SECTION II. EXPERIMENTS MADE FOR THE PURPOSE OF DETERMINING THE ELECTROMOTIVE FORCE OF THE SHOCK OF THE ELECTRICAL DISK.

In the course of the experiments made in 1887 at St Andrews, it was observed that the deflection produced by the discharge of the organ in response to excitation by a single induction shock, when the discharge was led through a galvanometer for a period of one five hundredth of a second including the moment of its maximum intensity, was about equal to that produced by 3 Callaud gravitation cells acting for the same time through the same circuit. Experiments were made at Plymouth for the purpose of obtaining more precise information on this subject.

The method consisted in balancing or compensating the electromotive force of the discharge by a measurable but variable electromotive force acting through the same circuit during the same time but in the opposite direction. In order that a method of this kind should give a true result, the closing time must be as short as possible and must include the period of culmination of the discharge. For this reason it can only be applied to a discharge of which the time-relations are accurately known. As an indicator the capillary electrometer already mentioned was used. This instrument was preferred because it was possible with its aid to make the observations much more rapidly than with any galvanometer we had at our disposal. The arrangement of the experiment is explained by the diagram.



In the diagram the horizontal line shews the course of the traveller. K_1, K_2, K_5 and K_6 having been previously closed and K_3 and K_4 opened, the traveller as it flies from left to right, opens K_1 and K_2, closes K_3 and K_4 and opens K_5 and K_6 in rapid succession. The strong arrows indicate the direction of the "organ-current," the weak ones that of the compensating current.

In order that both the wires connecting the electrometer with the contacts should be broken before and after the period during which it was to be acted upon by the discharging organ, it was necessary to provide in addition to the two keys K_1 and K_2 connected with the exciting circuit, the two other pairs of keys shewn in the diagram, viz. K_3 and K_4, K_5 and K_6. Of these K_3 and K_4 are closing keys, i.e. they are so constructed that the rapidly moving traveller liberates a spring which thereupon makes contact with a knob. In using such keys there is a loss of time which must be estimated empirically. For this purpose the zero position, i.e. the position in which the closing keys must be placed, in order that their simultaneous closure may coincide with the break of K_1, was determined by the telephone as follows:—The induction shock caused by the break of K_1 was led through a telephone, in the circuit of which the closing keys were interposed. The closing keys were then placed so that they were closed by the passage of the traveller before K_1 was broken, in which case the sound of the induction current can be heard telephonically as the traveller flies past. The closing keys were then shifted at each trial by little and little until the sound could no longer be heard. When this happens the keys are at zero. This method is so easy that it can be repeated as often as necessary. The keys K_5 and K_6 are break keys of the kind previously described.

It is seen that during the short period which intervenes between the simultaneous closure of K_3 and K_4 and the simultaneous opening of K_5 and K_6 the sulphuric acid terminal of the electrometer is connected through K_4 and K_6 with the leading off electrode next the distal end of the organ, and the capillary through K_5 and K_3 with the proximal electrode, and that before and after this period the wires from both terminals are broken. This is necessary in order that the instrument may not be influenced by escape from the battery circuit either before or after the period of closure.

In the diagram a Rheochord wire 303 centimeters long is represented at Rh.; and Gr. is a small Grove cell. By experiments at Oxford it was determined that when the circuit of the Grove was closed, the difference of potential between *a* and *b* was 1·587 Daniell. Consequently as the wire is divided into a hundred parts each division = 0·01587 Daniell. The arrangement of the exciting circuit (the wires of which are represented by fine lines) is such that immediately after the induction current has passed through the preparation, the secondary circuit is broken by K_2. If this were not done the leading off electrodes would be short circuited during the period of closure.

It was shewn in the former paper that the passage of an induction current through the separated organ is responded to by a discharge of which the greatest intensity is reached during the second hundredth of a second. If therefore the keys are so set that the closing time includes this period, the largest possible effect with a closure of this duration would be obtained. In all cases the organ was dissected out without injury to its surface, laid on a glass plate, and surrounded by two rings of kaolin paste made with 0·6 per cent. solution of chloride of sodium. To each ring two nonpolarisable electrodes were applied, one for leading in the exciting induction current, the other for leading off the response, as shewn in the diagram.

The primary circuit of the induction apparatus always contained, as above stated, one Grove's cell. The pair of closing keys K_3 and K_4 were at a distance from the two breaking keys, K_5 and K_6, corresponding to $\frac{1}{100}$ second. The interval of time between the opening of K_1 and the closing of K_3 and K_4 (i.e. between the excitation and the beginning of closing time) was also $\frac{1}{100}$ second. K_2 was broken half way between the two, i.e. $\frac{1}{200}$ second after the opening of K_1.

Experiment 1. *R. clavata* 24 inches long and 19 inches across; length of tail 12 inches. The difference of potential due to the "organ current" between the distal and proximal contacts (5 centimeters) was indicated by 7 divisions of the electrometer scale, its direction being +, i.e. that of the discharge as indicated in the diagram by the thick arrow. It was ascertained that the movement of the meniscus to which this difference gave rise with a closing time of $\frac{1}{100}$ second was less than a division of the scale. When the organ was excited without compensation, i.e. with the rheochord circuit open, the response was indicated by an excursion of over + 40 scale. When opposed by the derived external current, obtained by placing the slider of the rheochord at 90 (corresponding to a difference of potential of about 1·42 Daniell), the excursion was diminished to 20 scale; and to 9 scale when the slider was shifted to 100 (1·58 D.), i.e. to the furthest point of the rheochord wire. It was therefore necessary to take a shorter length of organ. For this purpose the distal kaolin ring was brought to 2½ centimeters distance from the proximal, and the experiment repeated. With the slider at 50 (0·79 D.) there was an excursion of + 6 scale; with the slider at 60 (0·95 D.), it was annulled. After each excitation there was the usual after effect (see previous paper Section VI.) so that it was necessary to allow a sufficient time to elapse between each observation and its successor for it to subside.

The distal ring was now removed to its first position and the proximal to that before occupied by the distal, so that they were still at a distance of 2½ centimeters. With the slider of the rheochord at 60, the direction of the excursion was reversed, shewing that the response was over-compensated. With the slider at 50, there was no movement. Adding the two results we have for 5 centimeters of organ a difference of potential during the second hundredth of a second after excitation, of at least 1·74 Daniell.

On counting the disks included in the whole tract, that is in 5 centimeters of organ, the number was found to be 80, this gives for each disk about 0·02 Daniell.

Experiment 2. R. clavata 30″ × 23″; length of tail 15″. Kaolin rings at a distance of 10 centimeters; "organ current" 12 scale of electrometer; this gave less than a division, with a closure of $\frac{1}{100}$″. The response with the same closure gave a large excursion, which when opposed by the derived current obtained by placing the slider at 75 (1·19 D.), was reduced to 20 scale: with the slider at 95 (1·5 D.) an excursion of 16 was obtained. With the position of the kaolin rings altered so as to include the proximal half of the same tract, the excursion was reduced to 6 scale: with the slider at 75 (1·19 D.) it was annulled. The distal half gave similarly an excursion which was annulled with the slider at 50 (0·79 D.). In this preparation therefore 5 centimeters of organ gave a difference of potential during the second hundredth of a second, of 0·99 D. On counting the disks, the number was found to be 66; this gives for each disk 0·015 D. Observations of the same kind were made on four other specimens, in all of which the electromotive force of the discharge was considerably lower, the mean being 0·66 Daniell for 5 centimeters of organ. As this is a case in which a mean does not represent the truth, it being certain that the functional activity of the separated organ even when prepared most successfully and from the most vigorous specimen, will be below that of the living organ, it is unnecessary to give these experiments in detail. Even if the organ were in full activity the result would still, from the nature of the method, fall short of the truth, for the fact which it represents is that the influence of the organ on the electrometer during a period of $\frac{1}{100}$ of a second is equal to that exercised by a known electromotive force acting during the same period. But inasmuch as this period includes both the culmination and the commencement of the decline of the electromotive force of each disk, it is obvious that the maximum electromotive force attained must be much greater.

All therefore that can be said is that the electromotive force of the response to a single excitation in each disk exceeds 0·02 Daniell.

In other experiments the organ was excited indirectly through the spinal cord after removing the tail as described in Section III. of our former paper. The electrodes were of course applied to the tail without any dissection.

In each experiment it was necessary first to ascertain the time-relations of the response, so as to select a closing time which would include its culmination. It was generally found that the 3rd hundredth (0·02″ to 0·03″ after excitation) satisfied this condition. The highest result which could be obtained when the discharge was led off by cables surrounding the tail at a distance of 10 centimeters from each other, was an excursion which was annulled by 55 division of the rheochord (= 0·87 Daniell). This gives 0·44 D. for five centimeters. In general the results were even lower than this, under 0·2 D. The explanation of the difference is principally, that the led off tract is only partially discharged.

Exhaustion of the Organ by previous activity.

In all electrical fish the electrical organ readily loses its functional activity by discharges. The method of compensation above described affords a means of proving that the loss of power has its seat in the organ itself. The following experiments relate to this point.

In a separated organ (R. clavata) the electromotive force of the response as led off from a tract (A) 2½ centimeters long, was found to be for the 2nd hundredth of a second after excitation, 0·79 D. and remained nearly unaltered during ten minutes. In the adjoining tract (B) of the same length, the corresponding result was 0·87 D. Tract A was then excited for 5 seconds, by the alternating induction currents of the inductorium (without side wire) the hammer vibrating 50 times per second. Time having been allowed for the partial subsidence of the after effect, the observation was repeated. The excitatory effect was now balanced by 0·39 Daniell. Tract B was found to be unaltered. It was now excited in the same way as tract A, after which the electromotive force of the excitatory response was reduced from 0·87 to 0·6 Daniell. After a quarter of an hour's interval, no recovery had taken place. It was then again excited and reduced thereby to 0·45 Daniell.

In order to observe the exhausting effect of the normal discharge, i. e. a discharge excited through the nervous system, the organ must be investigated *in situ*. In a large R. clavata (34 inches long by 23

wide) the discharge was led off from the tail by cables at a distance of 8·5 centimeters. Through these cables the organ was excited by a single induction shock, and gave an excursion of 27 scale when the electrometer circuit was closed during the second hundredth after excitation. It was ascertained in the same way as before, that this excursion was annulled by a length of rheochord wire corresponding to 0·48 Daniell. The organ was now discharged by electrical excitation of the optic lobes for two seconds at a time, as described in Section I.; and this was repeated ten times, each excitation evoking the usual discharges, after which the response to a single excitation was balanced by (0·16 Daniell). The spinal cord was then excited by alternating induction currents for similar periods, with the effect of further reducing the response to 0·06 Daniell.

In another skate the value of the response to a single induction current was 0·24 Daniell as led off from a tract 7·5 centimeters long. The spinal cord was divided behind the bulb and excited as before, each excitation producing the characteristic discharges. On reinvestigating the state of the organ it was found that the electromotive force of the response to a single excitation was reduced from 0·24 Daniell to 0·045 Daniell. This experiment was repeated in various ways, with the same results. No recovery took place in any instance while the observations were continued, but there can be no doubt, that if it were possible to maintain the normal conditions of circulation, recovery would eventually take place[1].

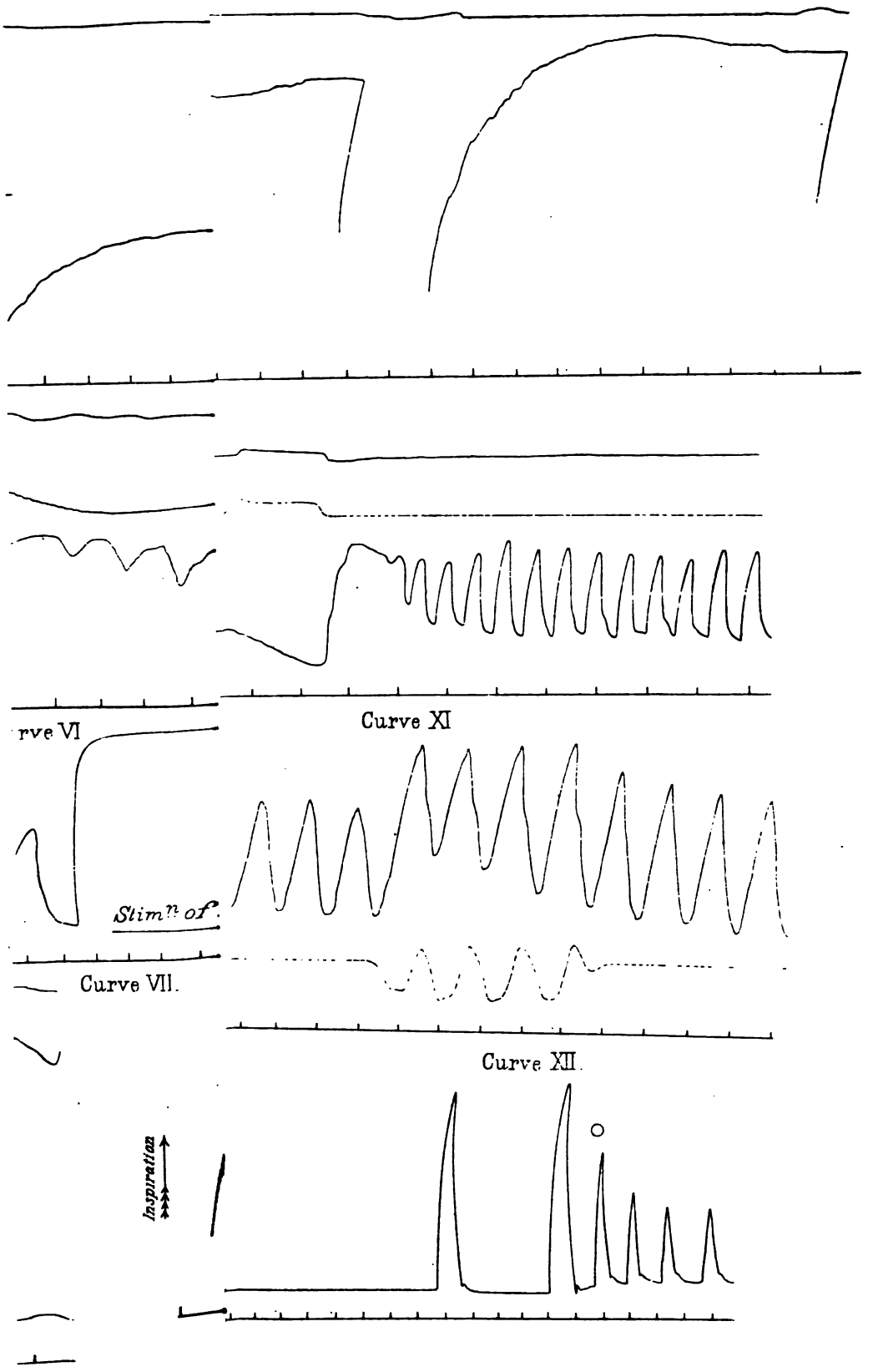
Conclusions.

1. Spontaneous discharge of the electrical organ of the skate has not, so far as we know, been observed. A reflex discharge can always be induced by mechanical stimulation of the skin, particularly that of the dorsal surface.

2. The afferent paths by which the reflex is excited are contained for the most part in the spinal nerves. Although discharges follow electrical excitation of certain branches of the trigeminus, they can be evoked reflexly, after all the cranial nerves have been divided.

3. The reflex discharge is always discontinuous, i.e. it consists of a group of two or more single electrical effects which follow each other with a frequency of from 8 to 25 per second. If the stimulation

[1] This might have been ascertained by exciting the organ *in situ* so as to exhaust it and then replacing the skate in the tank. It did not occur to us to do this.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Curve XII.
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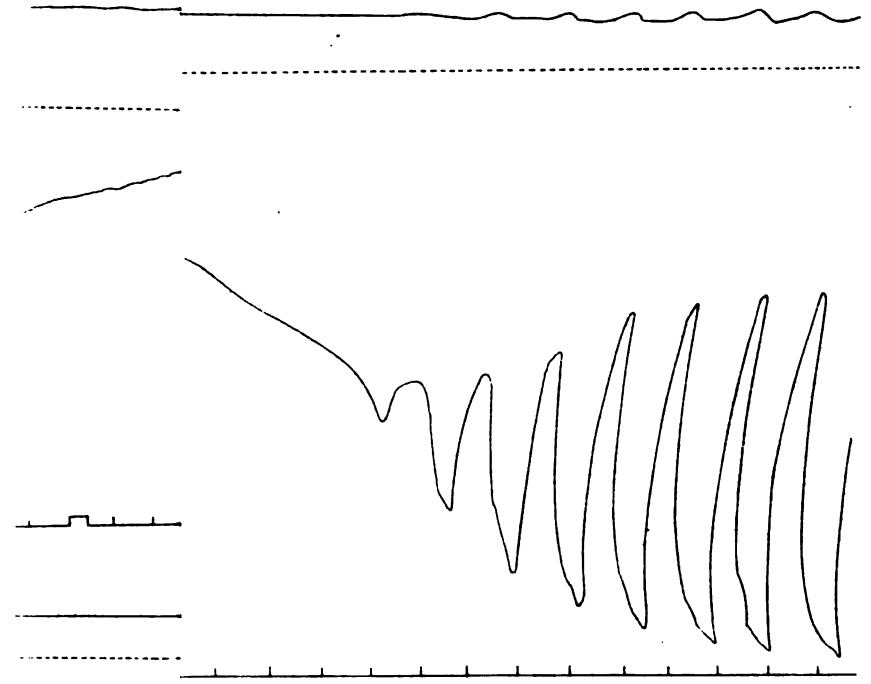
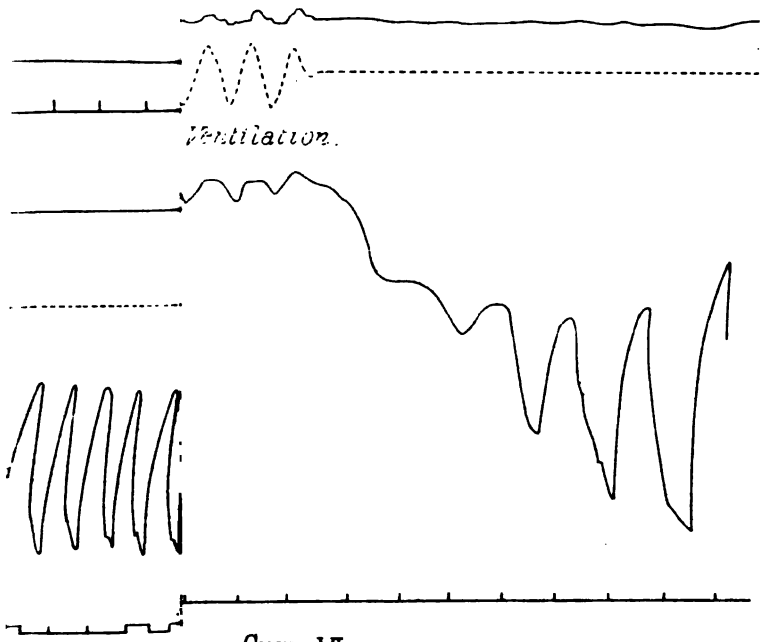
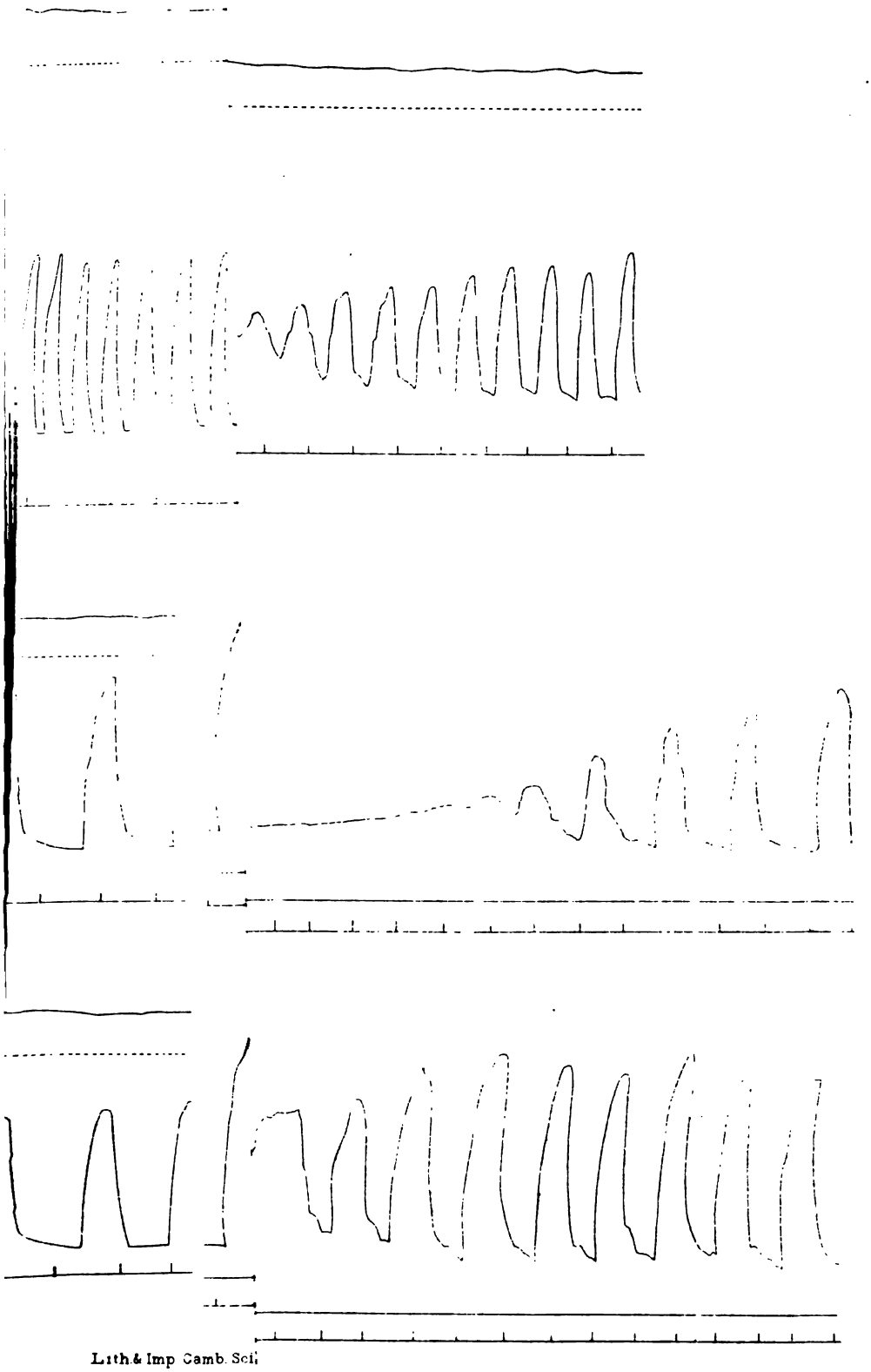




Curve VI.



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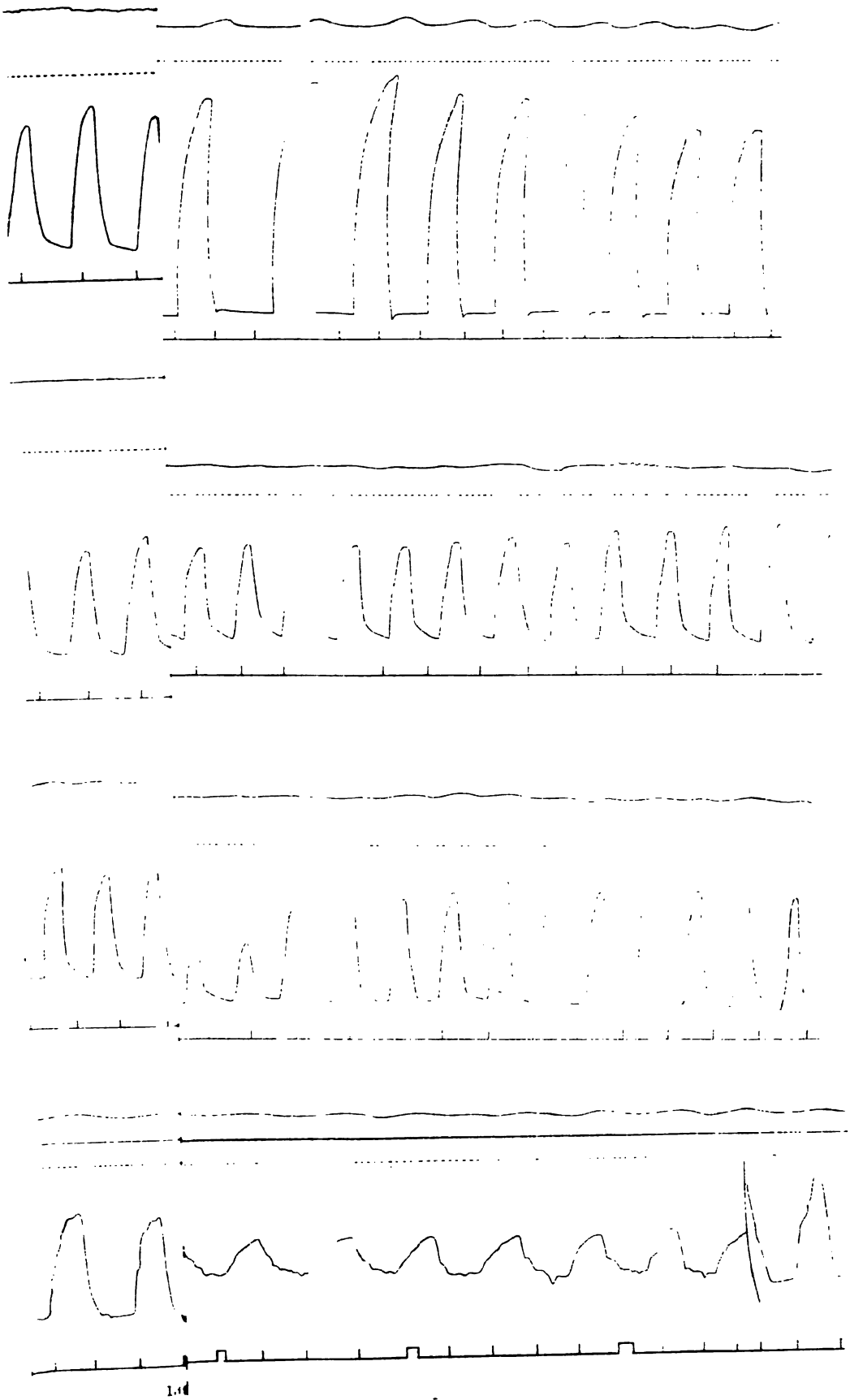

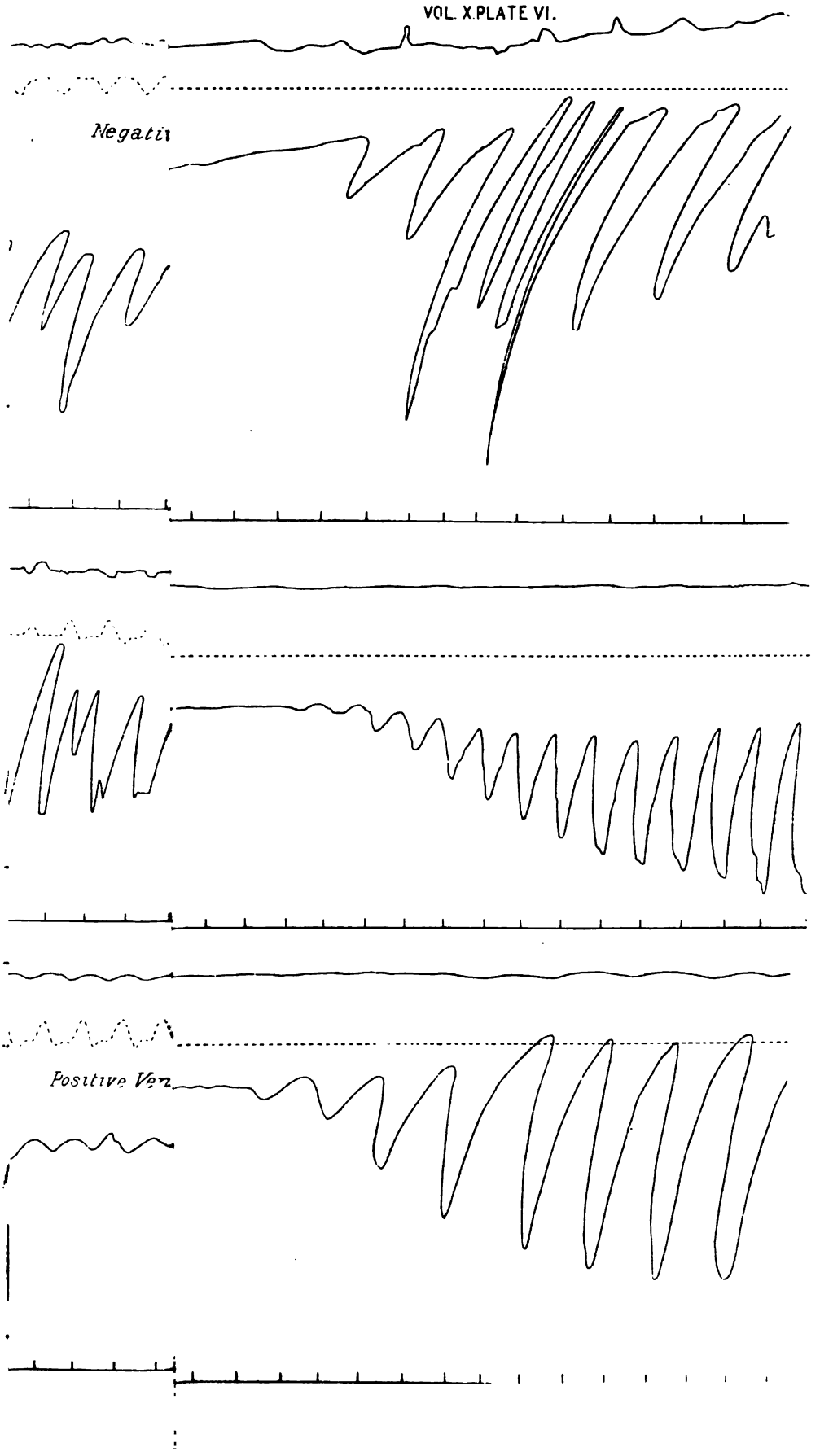
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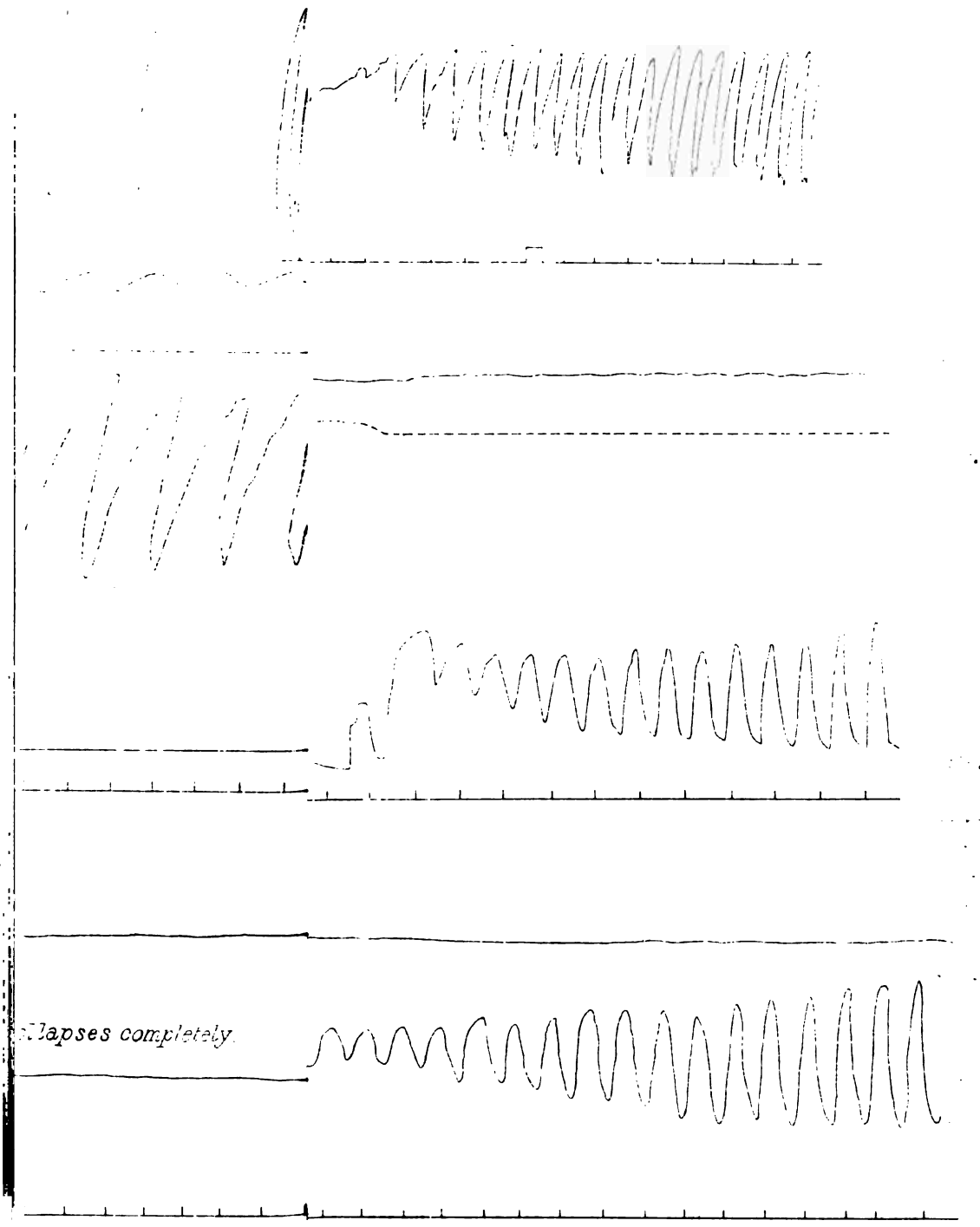
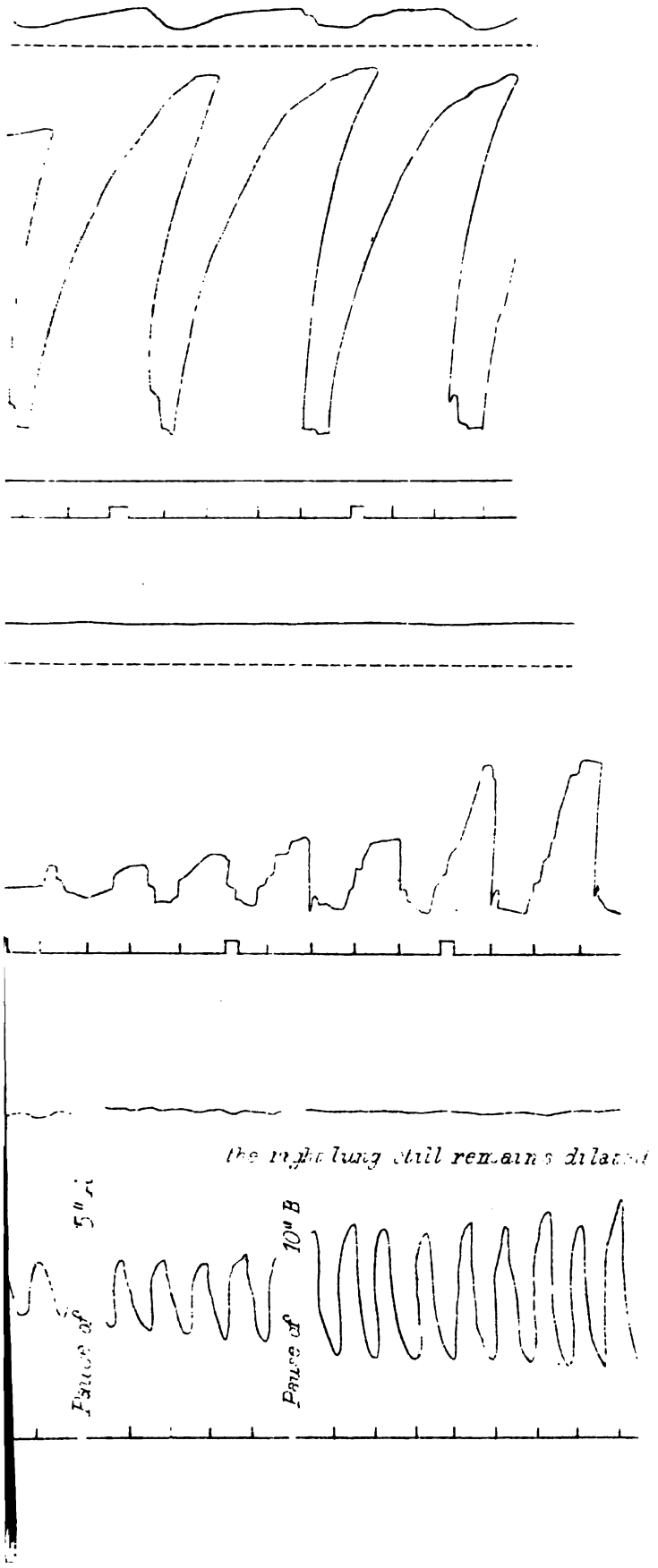

Collapses completely.


the right lung still remains dilated
Pause of 5" A
Pause of 10" B

is prolonged, this primary group may be succeeded by others of a similar character.

4. A reflex centre is situated in the optic lobes. Electrical stimulation of the dorsal surface of these lobes produces while it lasts a discontinuous discharge of the same character as the primary reflex discharge.

5. The discharge goes on after the excitation has ceased, manifesting itself either by prolongation of the primary effect, or by the recurrence at intervals of secondary effects similar to the primary.

6. Electrical stimulation of the anterior region of the cord after it has been separated from the bulb, also evokes a discontinuous discharge which lasts as long as the excitation. It is followed by effects of the same kind as those which follow excitation of the optic lobes, but these are less intense and of less duration. This part of the cord must therefore be capable of automatic action, although there is no evidence that it can be excited reflexly.

7. Each disk of the electrical organ is capable of developing during the state of excitation evoked by a single induction shock an electromotive force of over 0·02 Daniell. The sartorius muscle of the frog (as we have found in our own experiments) is capable, when excited by a single induction shock led through its nerve, of developing an electromotive force of 0·026 Daniell[1]. The number of nerve fibres distributed to the disk is certainly not less than that of the constituent fibres of the nerve of the sartorius. There is therefore no reason to regard the electrical activity of a disk as extraordinary as compared with a muscle of similar innervation.

The relatively large electromotive force which the organ as a whole is able to develop is attributable, (1) to the large number of disks of which it consists, (2) to their being arranged in pile, and (3) to the nervous arrangements by virtue of which they are enabled to act simultaneously.

[1] The experiments on the sartorius were made with the repeating rheotome. The estimate given above is probably too low.

EXPLANATION OF PLATE XXII.

Figs. 1 and 2. Rheoscopic curves produced by reflex discharge of organ of *Raia microcellata* following rubbing of dorsal skin.

Fig. 3. Similar curve produced by reflex discharge of organ of *Raia clavata* following rubbing of dorsal skin.

Figs. 4 and 5. Curves produced by discharge of organ of *R. maculata* following electrical excitation of optic lobes.

Figs. 6 and 7. Curves produced by discharge of organ of *R. clavata* following electrical excitation of spinal cord. The duration of the excitation is shown by the dotted line.

Figs. 8, 9 and 10. Curves produced by discharge of organ of *R. maculata* following salt excitation of optic lobes.

ON THE REGULATION OF RESPIRATION.

By HENRY HEAD, M.A.

(*From the Physiological Laboratory of the German University in Prague.*)

PART II. Theoretical[1].

If we attempt to take a general survey of the nervous mechanism of respiration we must begin by confessing that we are entirely ignorant of the cause of the rhythmic activity of the respiratory centre. Although the vagi play an important part in regulating the breathing they certainly are not the ultimate cause of rhythmic respiration; for rhythmic breathing still continues, although in an altered form, even after the vagi have been divided. Moreover the centre still sends out rhythmic impulses even when the medulla oblongata is separated from the rest of the brain, the spinal cord severed below the seventh cervical vertebra and the vagi, superior laryngeal and glossopharyngeal nerves divided. Now whatever may be the stimulus which keeps up the activity of the respiratory centre it is certainly not of a rhythmic nature, and we are brought face to face with the difficulty that a continuous stimulus produces discontinuous activity in the organ upon which it acts. So far we are unable satisfactorily to explain why this should be, but it is one of the earliest phenomena which meet us in the study of vital activity. Postulating however a primitive rhythmic activity in the respiratory centre, it is possible to examine the various modifications which it undergoes under the influence of stimuli produced by alterations in the volume of the lungs.

As soon as the lungs are inflated all inspiratory activity ceases and a prolonged pause follows, during which the rhythm of the centre is in abeyance and the inspiratory muscles in a condition of complete relaxation. In the same way complete collapse of the lungs also abolishes rhythmic breathing but the inspiratory muscles are now in a condition of strong tonic contraction. In both cases rhythmic breathing is sup-

[1] Part I, see p. 1.

pressed for a very considerable period in consequence of the sudden and permanent alteration in the volume of the lungs but under ordinary conditions it ultimately regains its rhythmic character in consequence of the growing dyspnoea. But even when precautions are taken to prevent dyspnoea, as in the experiments described in the previous section, the respiratory rhythm finally reappears even though the volume of the lungs remains unaltered. We must therefore conclude that the nervous mechanism of respiration possesses the power of adapting itself in some way or other to any volume which the lungs may permanently assume.

Now this adaptation might take place in one of two ways. Either the peripheral end organs of the vagus or the trunk of the nerve itself might become exhausted and so gradually cease to transmit the stimulus to the centre; or, the actual stimulus transmitted to the centre might remain practically constant and yet the gradual development of some antagonistic activity in the centre might lead to adaptation. Now there is little doubt that a stimulus passing up the vagi for a sufficient length of time would ultimately lead to the exhaustion of these nerves; but I think that a critical examination of the experiments, described in this paper, tends to show that this factor has little or nothing to do with the phenomena of adaptation under the conditions in which I have met with it.

Inflation of the lungs, under conditions which preclude any disturbance from dyspnoea, produces a long inhibitory pause in the breathing which is ultimately broken by an inspiratory contraction. Now if the end organs of the vagi or these nerves themselves gradually became exhausted we should expect that this inspiratory contraction would be very weak and would be followed after a long interval by a second contraction of somewhat greater strength. Thus, as exhaustion of the vagi proceeded, each inspiration would be stronger than that which came before it, and the pauses between these inspirations would gradually diminish. But as we saw in Part I. adaptation to a continuous inhibitory stimulus takes place in a very different manner. The pause, it is true, is ultimately broken by an inspiratory contraction, but far from being of diminutive size this contraction is actually stronger than that of any subsequent inspiration. Moreover the inspirations gradually decrease in strength and give place to a peculiar slow tonic contraction of the muscles of the diaphragm. The lever rises slowly, and finally remains for a short time steady at a certain height. Then small oscillations make their appearance and rhythmic breathing has

begun again although the lung remains in the same dilated condition as at the beginning of the experiment. Thus the actual modification which the breathing undergoes, during the time when the centre is becoming adapted to the dilated volume of the lungs, differs so greatly from that which we should expect if adaptation were due to a process of exhaustion in the nerve trunk, that I think we must assume that it is due to some change in the centre itself rather than to exhaustion of the peripheral mechanism.

Now if the centre possesses the power of adapting itself to any permanent volume of the lungs it is obvious that the sudden assumption of a certain medium volume will produce a different effect, according to whether the centre was previously adapted to a volume in excess of, or smaller than this medium volume. If the lungs have remained collapsed sufficiently long for adaptation to have taken place, a slight inflation up to a certain medium volume will produce an inhibitory stimulus and cause the cessation of all rhythmic inspiratory activity. If on the other hand the centre is adapted to a dilated condition of the lungs the sudden assumption of the same medium volume will produce a strong inspiratory effect. Thus the nature and extent of the influence produced by a certain volume of the lungs depends not only on the actual volume but also on its relation to the total volume to which the centre has become adapted.

Some physiologists are accustomed to regard inhibition, whether central or peripheral, as a decrease in the vitality of the organ whose external activity has been checked. The clearest expression of this idea is found in the statement that stimulation of the vagus causes stoppage of the heart by lowering its vitality. It is further assumed that this is the only effect produced by an inhibition, and the heart is said to be thrown into a condition of complete rest. But of late years many observers[1] have called this view of peripheral inhibition in question, and I think that an examination of the experiments in this paper tends to show that it is insufficient to account for the phenomena of inhibition in the respiratory centre.

If the lungs are inflated and allowed after a few seconds to return to their normal volume, I pointed out that the pause so produced was frequently followed by the clearest traces of increased inspiratory activity. The diaphragm was more or less under the influence of a tonic inspi-

[1] Especially Hering, Gaskell and Biedermann.

ratory innervation of which all traces had been previously absent. If we assume that the pause in the breathing produced by the inflation was caused simply by a diminution in the vitality of the respiratory centre we should expect that the breathing would return to the normal type as soon as the lungs returned to their normal volume. If we attempt to explain the increased inspiratory activity, which appears when the inflation is over, by the assumption that the centre has become dyspnoeic during the inhibitory pause, it is difficult to see why the respiratory rhythm should be so profoundly altered. For, provided the vagi are intact, dyspnoea is characterised by strong inspiratory contractions noticeable for their comparatively short duration and for the completeness of the expiratory elongations by which they are followed. On the other hand the increased inspiratory activity, which follows the close of an inflation, is characterised by the prolonged duration of the inspiratory contractions and the incompleteness of the expiratory elongations. The expiratory period may be so shortened that, although the rhythmic contraction of the inspiratory muscles scarcely reaches the height of a normal inspiration, it is followed by the most distinct traces of a tonic contraction of the inspiratory muscles. Moreover these phenomena are seen to follow the close of an inflation with hydrogen almost as readily as when the oxygen has been the medium of inflation. Thus the expression of the increased inspiratory activity which follows the return of the lungs to their normal volume after an inflation is in every way different from the inspiratory changes in the breathing caused by dyspnoea; for the experiments described in this paper have repeatedly shown that dyspnoea caused by deficient oxydation is especially inimical to the development of an inspiratory tone.

We also saw that an inspiratory stimulus of known intensity gained additional activity if preceded by an inflation. Thus electrical stimulation of the central end of one vagus, which was not sufficiently strong to completely abolish the respiratory rhythm and produce Rosenthal's "tetanus," was enabled to cause this characteristic inspiratory contraction if preceded by an inflation.

Again under certain conditions negative ventilation is no longer able to completely annihilate rhythmic breathing, much less to produce an apnoea pause after the ventilation has ceased. If under such conditions the lungs are inflated for a few seconds during the negative ventilation, rhythmic breathing ceases under the influence of the next few strokes of the pump and cessation of ventilation is followed by the

usual apnoea pause so characteristic of negative ventilation. Thus a rhythmic inspiratory stimulus which was unable to overcome the rhythmic activity of the respiratory centre sufficiently to produce a tonic inspiratory apnoea regains its full power when preceded by an inflation.

All these experiments tend to show that an inhibitory stimulus from the lungs not only diminishes the inspiratory energy expended by the centre but also in some way increases its potential inspiratory energy. Now Gad[1] has already pointed out in his very suggestive paper that there is no reason on analogy to suppose that a tissue can be brought to a condition of complete rest by a stimulus but that on the other hand we know of a number of phenomena in cell life which show that the form assumed by the activity of the cell can be altered. He applies this idea in detail to the respiratory centre and points out that, whereas the one stimulus might increase the expenditure, the opposite stimulus might turn the energy of the centre in the direction of nutrition or of some other activity during which no stimulus passes from the ganglion cells to the fibres of the motor nerves. He suggests that whereas inspiratory stimuli tend especially to increase the destructive activity of the respiratory centre, inhibitory stimuli may possibly increase its constructive or anabolic activity.

Now assuming for the moment that an inhibitory stimulus produces a preponderating constructive activity in the respiratory centre, it is obvious that the longer it acts the greater will be the amount of material stored up in the centre which, on breaking down, would give rise to inspiratory energy. But the greater the amount of such material stored up in the centre the less becomes the irritability of that centre for the inhibitory stimulus; for the irritability of a centre for any particular stimulus is inversely proportional to the amount of change that stimulus has already produced. In other words, the greater the amount of material collected in the centre the harder it becomes to add permanently to that amount. Finally a stage must be reached in which the amount of constructive activity due to the inhibitory stimulus during any given period, exactly equals the expenditure of inspiratory energy in the same time and adaptation is complete. But although the constructive and destructive activity of the respiratory centre are now equal as at the beginning of the experiment, the condition of

[1] "Ueber die in der Lehre von der Regulirung der Athemthätigkeit angewandte Terminologie." Würzburg, 1880.

the centre is in reality very different after adaptation has taken place from that which existed before it came under the influence of the continuous inhibitory stimulus. What might be called the "vitality" of the centre has been greatly increased, for it is now in a condition to respond with far greater vehemence to an inspiratory stimulus; moreover the slightest diminution in the inhibitory stimulus would at once lead to the liberation of inspiratory energy, apart from any inspiratory stimulus—in fact the whole level of activity has been shifted upwards. For, whereas before the centre came under the influence of the inhibitory stimulus it expended at any moment the same amount of energy as it accumulated and this had no balance upon which to fall back, it now has a store sufficient to meet considerable calls, in consequence of the accumulation which took place during the time when its constructive exceeded its destructive activity.

When Hering and Breuer proved that collapse of the lungs caused an inspiratory effect they assumed that diminution in the volume of the lungs produced an actual inspiratory stimulus analogous to the inhibitory stimulus caused by an inflation. Gad, on the other hand, explains the undoubted inspiratory effect produced by collapse as due rather to the complete removal of the inhibitory stimuli which normally ascend the vagi to act on the mainly inspiratory centre. It is extremely difficult to decide between these two views; for, as I pointed out above, the removal of a permanent inhibitory stimulus causes a great outburst of inspiratory energy, apart from the presence of any actual inspiratory stimulus. Then again, though the respiratory centre is not originally mainly inspiratory, as assumed by Gad, yet it undoubtedly becomes so under the continuous action of an inhibitory stimulus. We must therefore keep clearly in view the possibility that collapse of the lungs may produce two distinct effects both tending to modify the breathing in the same manner. Thus diminution in the volume of the lungs may evoke, firstly, the strongly inspiratory negative afteraction due to the removal of an inhibitory stimulus, and, secondly, an actual inspiratory stimulus analogous to the inhibitory stimulus produced by a dilatation of the lungs. Now as far as my experiments go towards solving this very difficult problem, the following reasons incline me rather to the original view of Hering and Breuer that diminution of the lungs below their normal volume is an actual inspiratory stimulus to the respiratory centre.

If both lungs are allowed to collapse suddenly by puncturing the thorax a much stronger inspiratory effect is produced than if both vagi were divided and yet, according to Gad's hypothesis, both operations equally remove the inhibitory stimuli normally passing up the vagi from the lungs. Again, a momentary suction will often produce a stronger inspiratory contraction than any which appears after the vagi are divided, even at the moment of division when the vitality of the centre cannot have materially fallen. Then too the very fact that a single momentary diminution in the volume of the lungs causes an effect which lasts after the actual diminution is past and which, when periodically repeated (negative ventilation), can undergo summation speaks in favour of an actual inspiratory stimulus.

I think it is difficult to explain these differences unless we assume that collapse of the lungs below the normal volume produces an actual inspiratory stimulus. On the other hand it would appear as if the normally diminished volume which the lungs assume when the chest is at rest, produces no stimulus to the end organs of the vagi although the sudden assumption of this same volume may produce an inspiratory or expiratory modification in the breathing if the centre was previously adapted to a permanently increased or decreased volume of the lungs. This hypothesis is borne out by the phenomena which follow closure of the trachea in the expiratory phase of breathing under certain special circumstances. For as we have already seen closure of the trachea in the expiratory phase in a normal animal greatly modifies the inspiratory portion of the breathing but leaves the expiratory phase almost unaltered; the inspirations are increased both in strength and duration and assume an appearance closely resembling that seen after division of the vagi. But if one side of the thorax has been opened and the vagus of the opposite side divided, closure of the trachea produces no alteration in the breathing provided each vagus only supplies the lung on its own side of the thorax, and in those cases where an alteration occurs we can be certain that there is some crossed innervation. Now if closure of the trachea in the expiratory phase does produce an alteration in the breathing under such conditions, not only are the inspiratory contractions increased in strength as was the case in the normal animal but the expirations are also shortened by the appearance of an inspiratory tone (Curve xii. Plate 1). This new factor cannot be explained on the hypothesis that inhibition is now more completely removed than was the case in the normal animal, for subsequent division of the intact vagus does not cause the appearance of a tonic

inspiratory contraction under these conditions. Now if this tonic inspiratory contraction were due to contrast evoked by the sudden removal of all inhibition it ought equally to appear when all possibility of inhibition is suddenly removed by freezing the remaining vagus. So far I think the difference in the results obtained in the two cases can only be explained by supposing that the abnormally collapsed lung causes an actual inspiratory stimulation to the vagi which is set free to act as soon as the trachea is closed in the expiratory phase and which is removed, together with the inhibition, by the division of these nerves.

What is the nature of this inspiratory stimulus? We have already seen ground for supposing that an inhibitory stimulus caused by dilatation of the lungs increases the constructive activity of the centre, and on analogy we should be inclined to assign an opposite function to the inspiratory stimulus caused by abnormal collapse of the lungs. The following experiment, though not in any way conclusive, seems to throw some light on this question. If the brain has been separated from the medulla oblongata and both lungs are allowed to collapse a strong inspiratory tone is produced which gives place, as dyspnoea advances, to strong rhythmic inspirations accompanied by an ever decreasing tonic contraction of the inspiratory muscles. In the course of a few minutes the animal dies of dyspnoea if artificial respiration is not resorted to. But if both vagi are frozen through soon after the collapse of both lungs all inspiratory breathing ceases at once and a gigantic active expiration is developed, which slowly diminishes in strength to end in the death of the animal. Now as we have already seen division of both vagi after the medulla oblongata has been separated from the rest of the brain normally produces a very strong inspiration, which lasts as much as 40 sec. Thus the fact that division of the vagi was preceded by collapse of both lungs has fundamentally altered the result produced by division of these nerves; for, instead of a long inspiration, division is now followed by a strong active expiration. This strong expiration is not due to the dyspnoea necessarily caused by the collapse of the lungs, for the animal can be kept alive during the expiration by artificial ventilation and then allowed to die of pure dyspnoea by stopping the ventilation; death under these conditions is not characterised by any such violent expiration. These anomalous results can however be explained by assuming that before division of the vagi a powerful inspiratory stimulus from the collapsed lung was driving the centre to the utmost of its power, and that the

exhausted centre flew to the opposite extreme as soon as the inspiratory stimulus was removed, just as in a normal animal an outburst of inspiratory energy follows the removal of the inhibitory stimulus from the periodically dilated lungs.

Thus the inspiratory stimulus caused by diminution in the volume of the lungs would appear to be the true complement of the inhibitory stimulus caused by their dilatation. The inhibitory stimulus checks the inspiratory expenditure of the centre and at the same time stimulates it to greater constructive activity, thus tending to raise the level of its vitality. On the other hand the inspiratory stimulus would greatly increase the inspiratory expenditure and, by decreasing the constructive activity, tend to lower the level of vitality in the respiratory centre. Both stimuli tend indirectly to increase the irritability of the centre for the antagonistic stimulus and thus, provided sufficient time be allowed to elapse, adaptation to either form of stimulus must ultimately take place. When adaptation is complete rhythmic respiration will begin again, but the continuance of the stimulus is necessary to maintain the equilibrium of the centre; for its sudden removal at once throws the centre into the opposite form of activity (negative afteraction) without the intervention of any antagonistic stimulus.

When the vagi are divided the breathing finally settles down into a type characterised by long powerful inspiration; each inspiratory curve is broad and flat topped, and is terminated by a rapid fall of the lever, which may reach the base line of complete elongation. Here we have a form of rhythmic respiration entirely independent of any reflex regulation from the lungs, and it is interesting to consider the effect which would be produced by suddenly reinstating the vagi. The centre would send out a certain amount of inspiratory energy to produce one of the characteristic strong inspirations and the lungs would begin to dilate. But before the inspiration was half exhausted the dilatation of the lungs would cause an inhibitory stimulus and would check the inspiratory activity of the centre, the inspiratory muscles would elongate and a pause would be produced; for we saw that a momentary dilatation of the lungs not only checked the inspiration upon which it was superposed but also tended to hinder the production of the next inspiration. As soon as this positive afteraction was sufficiently diminished a second inspiration would appear, which would also be checked before it had reached its full height and would again cause an inhibitory afteraction.

Thus each inspiration is cut short by the dilatation of the lungs which it produces, and helps to add to the inhibitory condition of the respiratory centre. In addition each such inhibitory stimulus adds to the potential inspiratory energy of the centre and thus raises the vitality. Now as the store of inspiratory energy increases, the respiratory centre gradually becomes less irritable for the inhibitory stimuli, for the irritability of the centre for any particular stimulus is inversely as the change which that stimulus has produced. Finally a mean would be struck between the increased inspiratory energy of the centre and the periodic inhibitions from the periphery. The breathing would remain rhythmic but the inspiratory contractions would be smaller and more frequent than when the vagi are divided. Moreover the centre would be in a condition of increased vitality and would respond with much greater promptitude to an inspiratory stimulus or to a diminution in the inhibition, such as would be produced by dividing the vagi. Thus when the vagi are intact the centre has a constant tendency to exhibit inspiratory activity, whilst at the same time this inspiratory activity is checked and regulated by the dilatation of the lungs which it must necessarily produce. In fact we might now speak of the centre as being in a condition of "high level vitality." On the other hand when the vagi are divided the inspirations are no longer checked by the dilatation of the lungs, and are therefore of great strength and abnormal duration. If the centre is in a condition of high vitality at the time the vagi are divided the breathing at once becomes principally inspiratory, and the strong inspirations are accompanied by a permanent tonic inspiratory contraction during the expiratory phase. But later on the expirations gradually lengthen and the tonic inspiratory contraction disappears; this may occur immediately the vagi are divided if the vitality of the centre has been lowered by an excessive dose of chloral or some other maltreatment. Ultimately the centre settles down to a condition of spendthrift activity, regulated only by the impulses from the centres in the higher parts of the brain. Lastly, if the influence of these higher centres be removed by dividing the medulla oblongata just above the striae acusticae, all regulation disappears and enormous inspiratory contractions, which gradually decrease in duration, accompanied by ever increasing expiratory pauses quickly lead to the death of the respiratory centre from inanition.

During normal breathing the activity of the vagi produces two results—firstly, the inspiratory activity of the centre is regulated by

the constant inhibitions produced by the dilatation of the lungs, and secondly, the potential inspiratory energy of the centre is raised. Thus division of the vagi will also produce a twofold effect upon the breathing. In the first place the inspiratory activity of the centre is no longer checked by the dilatation of the lungs, and the inspirations therefore increase both in strength and duration. Now if this was the only effect produced by dividing the vagi the breathing would at once assume a regular form, characterised by strong inspirations of considerable duration, followed by complete expiratory pauses. If the vitality of the centre is lowered by an excessive dose of chloral this is actually the case, but, if the centre is in a presumably normal condition, the inspiratory contractions which immediately follow division of the vagi differ greatly from those which appear subsequently. The first inspiration which follows division normally greatly exceeds its fellow in duration, and the tonic contraction of the diaphragm is always most marked between the first and second inspirations. The excessive duration of the first inspiration and the presence of inspiratory tone are ultimately connected with one another, for they are both abolished by a large dose of chloral, by dyspnoea or by anything which tends to lower the vitality of the respiratory centre. They are not primarily due to the condition of the air in the lungs, for they may be entirely absent although the animal is breathing an atmosphere of pure oxygen, provided the vitality of the centre has been depressed by an excessive dose of chloral. During dyspnoea both phenomena are absent although division of the vagi causes a great increase in the strength of the rhythmic inspiratory contractions. On the other hand the difference between the duration of the first and second inspirations can be increased by dividing the vagi during an inflation, even if the lungs have been inflated with hydrogen. Now in this case the inhibition which was removed on dividing the vagi has been increased, and, as a consequence of this increase, the difference between the duration of the first and subsequent inspiration is more marked than usual. In the same way we have already seen that, when the vagi are divided during an inhibitory apnoea caused by periodic inflations, the first inspiratory contraction after the pause was noticeable for its enormous duration. Thus we must assume that division of the vagi not only removes a check upon the rhythmic inspiration, but also upsets the balance of the centre by setting free the potential inspiratory energy stored up during the time the centre was adapted to the inhibitory stimulus.

In conclusion, the vagi produced two effects upon the centre during

normal breathing. Firstly, each inspiratory contraction is checked when it has reached a certain height owing to the stimulus caused by the dilatation of the lungs, and secondly, the inspiratory vitality of the centre is increased owing to these frequently repeated inhibitory stimuli. Removal of these nerves will therefore produce two distinct modifications in the breathing, one of which is permanent, the other more or less transitory. Firstly, the inspiratory contraction will be increased in strength and duration in consequence of the regulating influence caused by the variations in the volume of the lungs. Secondly, the removal of the inhibitory stimuli will set free the greater part of the inspiratory energy stored up in the respiratory centre under the influence of this inhibition, and will cause the appearance of an inspiratory tone, which is most marked immediately after division of the vagi, and gradually passes off as the centre becomes exhausted.

In conclusion it is my pleasant duty to offer my sincerest thanks to Professor Hering for the extremely kind way in which he not only placed his laboratory at my disposal, for nearly two years and a half, but also helped me by every means in his power.

ON THE PHYSIOLOGY OF THE SALIVARY SECRETION. By J. N. LANGLEY, M.A., F.R.S., *Fellow and Lecturer of Trinity College, Cambridge.*

Part V. The effect of stimulating the cerebral secretory nerves upon the amount of saliva obtained by stimulating the sympathetic nerve.

In the summer and autumn of 1887, I was carrying on a series of observations upon the morphological elements in saliva. In these observations a few drops of saliva were obtained by stimulating the chorda tympani, or the sympathetic nerve, and the chemical nature of any bodies that could be seen in the saliva were tested under the microscope. Thus, now one nerve, now the other, was stimulated at very irregular intervals. Stimulation of the sympathetic gave at different times very unequal quantities of saliva, but, perhaps because such an inequality had often been noted before, by others as well as by myself, I paid no attention to it, until one experiment in which I wished to see whether repeated alternate stimulation of the chorda and of the sympathetic would alter the appearance of the spheres and lumps of sympathetic saliva. After obtaining several specimens, it struck me, that the sympathetic stimulation had without exception produced a flow of saliva, more abundant and less viscid than usual. I at once proceeded to try the effect of varying the pause between stimulation of the chorda and stimulation of the sympathetic, and it soon appeared that the sympathetic secretion was, broadly speaking, the more abundant the more quickly it was obtained after stimulation of the chorda. Having thus, somewhat accidentally, come upon this fact, and having satisfied myself that the sub-lingual gland gave the same results as the sub-maxillary, I naturally turned with much interest to the parotid gland of the dog, which had so rarely been found to secrete on stimulation of the sympathetic nerve. I obtained, what I had scarcely ventured to expect, a secretion from the gland following without fail on each stimulation of the sympathetic nerve, provided that Jacobson's nerve had been stimulated a short time previously.

A preliminary communication[1] on the subject I have already made, and I propose now to treat it more fully.

It will be convenient to take the sub-maxillary and parotid glands separately. With the sub-lingual gland I have not made so many nor such detailed observations as with either of the others, but the results obtained correspond so closely with those to be set forth for the sub-maxillary gland, that it is, I think, unnecessary to do more than simply to state that this is the case.

THE SUB-MAXILLARY GLAND.

Method of experimentation.

The following procedure was as a rule adopted; when in any experiment a divergence in procedure worthy of note was made, it is mentioned in the account of that experiment.

Morphium hydrochlorate was injected sub-cutaneously, generally in considerable quantity, viz. 2 to 4 c.c. of a 4 or 5 p.c. solution. In half-an-hour to an hour, chloroform if necessary being given, a three-way tube was tied in the trachea, and one limb of the tube connected with a bottle containing a mixture of chloroform and ether; the bottle was removed if the respiration became feeble.

The chordo-lingual nerve was cut and the chorda dissected out for a ¼ to a ½ an inch.

The sympathetic nerve was dissected out from the vagus in the neck for about an inch, ligatured and cut; usually the vagus was also ligatured and cut.

The cannula tied in Wharton's duct was connected by a three-way tube with a glass tube lying over a scale graduated in millimetres. When noting the rise of saliva in the tube, it was placed at an angle of about 30° from the horizontal. An assistant either noted the rise of saliva in the tube at definite intervals, as 30 secs., or stimulated the nerves as directed. Two tubes were used; 35½ millimetres of the larger, and 76 millimetres of the smaller, corresponding to ·25 c.c. The larger tube was used for the sub-maxillary gland except in Exps. IV., V. and VI.

One Daniell's cell was used with a du Bois Reymond's induction coil, arranged for an interrupted current. The position of the index of

[1] "On 'secretory' fibres in the sympathetic nerve supplying the parotid of the dog." *Proceedings Physiological Society*, No. 1, Feb. 11, 1888 (this *Journal*, Vol. IX.).

the secondary coil is indicated by $c = -$, thus $c = 9$ means that the index of the secondary coil was 9 c.m. from the primary. The position of the secondary coil is omitted when it was the same as that of the previous stimulation. Thus in Exp. II. the first three stimulations were with the secondary coil at 9 c.m. from the primary, and the rest of the stimulations with the secondary coil at 8 c.m.

In the experiments, the flow of saliva in millimetres was usually taken during each successive 30 seconds; in the account of the experiments the numbers representing the flow in millimetres in successive 30 seconds are written in a line; sometimes the flow of saliva was taken at shorter or longer periods, in such cases the time is placed above the number representing the rise of saliva during it; when during one of the periods, the chorda tympani or the sympathetic was stimulated, it is shown by putting Ch. or Sy. under the number representing the flow of saliva during the period. Thus in Exp. I.

$$\frac{1\tfrac{1}{2} \,.\, 1\tfrac{1}{2}}{\text{Sy.}} \,.\, \overset{7'}{3}$$

indicates that the sympathetic was stimulated for 60 secs.; that the saliva rose $1\frac{1}{2}$ mm. in the tube in the first 30 secs., and $1\frac{1}{2}$ mm. in the second 30 secs. and that the rise of saliva in the subsequent 7 minutes was 3 mm.

General Features.

I will first give some experiments to show the extent and duration of the effect of stimulation of the chorda tympani upon the subsequent stimulation of the sympathetic nerve.

EXPERIMENT I.

Oct. 29, 1887. Dog. Weight $5\frac{3}{4}$ kilos. Chorda tympani isolated but not cut. The same strength of stimulus was used throughout, viz. an interrupted current just felt to the tip of the tongue. V.S. below indicates that the combined trunk of the vagus and sympathetic was stimulated on the superior cervical ganglion side of the place at which the sympathetic was dissected out. Cannula in Wharton's duct connected with graduated tube, the rise of saliva in this in millimetres is given for every 30 secs. [except in (2) in which it is given for every 15 secs.] unless otherwise mentioned.

(1) 1.2. $0 . \underbrace{1\tfrac{1}{2} . 1\tfrac{1}{2}}_{\text{Sy.}} . \overset{7'}{\overline{3}} . \overset{1'}{\overline{124}} . 4 . 0 . \underbrace{15 . 4\tfrac{1}{2}}_{\text{Sy.}} . \overset{2'}{\overline{3\tfrac{1}{2}}} . \overset{8'}{\overline{0}} . \underbrace{1\tfrac{1}{2}}_{\text{V.S.}} . \overset{1'}{\overline{8}} . \overset{1'}{\overline{15\tfrac{1}{2}}} . \overset{7'}{\overline{0}} . \underbrace{\tfrac{1}{2}}_{\text{V.S.}}$

$9\tfrac{1}{2} . 2 . 2 . 1 . \tfrac{1}{2} . \underbrace{\overset{10''}{\overline{12\tfrac{1}{2}}}}_{\text{Ch.}} . \overset{90''}{\overline{10}} . \underbrace{\overset{15''}{\overline{22}} . \overset{15''}{\overline{5}}}_{\text{V.S.}} . 4 . 3 . 2 . 0$

(2) 2.2. Rise of saliva in successive 15 secs.

$\overset{5'}{\overline{0}} . \underbrace{\overset{1'}{\overline{1}}}_{\text{Sy.}} . \tfrac{1}{2} . \underbrace{6}_{\text{Ch.*}} . \underbrace{13\tfrac{1}{2} . 13 . 2\tfrac{1}{2} . 2}_{\text{Sy.}} . \overset{1'}{\overline{\tfrac{1}{2}}} . \overset{4'}{\overline{0}}$

* The stimulation of the chorda was stopped as soon as the secretion of saliva began.

(3) 2.13. $0 . 0 . \underbrace{5}_{\text{Ch.*}} . 1 . 0 . \underbrace{15}_{\text{Sy.}} . 1 . 0 . 0 . 0 . \underbrace{1\tfrac{1}{2}}_{\text{Sy.}} . \tfrac{1}{2} . \underbrace{7\tfrac{1}{2}}_{\text{Ch.*}} . 0 . \underbrace{\overset{15''}{\overline{9\tfrac{1}{2}}} . \overset{15''}{\overline{4}}}_{\text{Sy.}}$

* The stimulation of the chorda was stopped as soon as the secretion of saliva began.

(4) 2.24. Stimulate chorda for 2 min.; tube filled = 225 mm.

2.27. Stimulate chorda for 2 min.; tube filled = 225 mm., secretion continues.

2.30. $9 . 8 . 8 . 7 . \underbrace{\overset{15''}{\overline{13}} . \overset{15''}{\overline{2}}}_{\text{Sy}} . 4 . 2\tfrac{1}{2} . 2 . \underbrace{\overset{15''}{\overline{5\tfrac{1}{2}}} . \overset{15''}{\overline{3\tfrac{1}{2}}}}_{\text{Sy}} . \overset{2'}{\overline{5\tfrac{1}{2}}} . \overset{1'}{\overline{2}}$

(5) 2.48. $\underbrace{7}_{\text{V.S.}} . 3\tfrac{1}{2} . 4\tfrac{1}{2} . 4 . 4 . 3 . 2 . \underbrace{5}_{\text{V.S.}} . 2 . 2 . \underbrace{5}_{\text{V.S.}} . 2 . 3 . 2 . 2 . \underbrace{6}_{\text{Sy.}} . 0 . 0$

$0 . \underbrace{2}_{\text{Sy.}} . 0$

The vagus dissected out and stimulated gave no constant result, usually producing no secretion; possibly the effective portion of it was left behind with the sheath.

EXPERIMENT II. (*a*).

Jan. 2, 1888. Dog. Weight $6\tfrac{1}{4}$ kilos. The secondary coil at 9 c.m. gave a current feebly felt on the tongue. The same coil and electrodes were used for both chorda and sympathetic.

(1) 12.30. Stimulate chorda, secretion continues slowly after stimulation.

12.37. Rise each 30 secs. $2 . 2 . 1\tfrac{1}{2} . 1\tfrac{1}{2} . \tfrac{1}{2} . \underbrace{4\tfrac{1}{2}}_{\text{Sy. } c=9} . 2\tfrac{1}{2} . 2 . 1 . \underbrace{\overset{15''}{\overline{9\tfrac{1}{2}}}}_{\text{Ch.}} . 9$

$1 . \underset{\text{Sy.}}{5\tfrac{1}{2}} . 1\tfrac{1}{2} . 1 . 1 . 1 . 1 . 1 . \tfrac{1}{2} . \underset{\text{Sy. } c=8}{2\tfrac{1}{2} . \tfrac{1}{2}} . 1 . \tfrac{1}{2} . \underset{\text{Sy.}}{3} . \tfrac{1}{2} . 0 . \overset{15''}{\underset{\text{Ch.}}{7\tfrac{1}{2}}}$

$\overset{15''}{10} . 5 . 2 . \underset{\text{Sy.}}{11} . 1\tfrac{1}{2} . \tfrac{1}{2} . \underset{\text{Sy.}}{2} . 1 . \underset{\text{Ch.*}}{11} . 3 . \underset{\text{Sy.}}{\overset{15''}{6} . \overset{15''}{2}}$

* Chorda stimulated for five seconds.

(2) 12.58. $\overset{90''}{\underset{\text{Ch.}}{180}} . 7 . 1 . \underset{\text{Sy.}}{\overset{15''}{16} . \overset{15''}{7}} . 5 . 0$

(3) 1.7. $\underset{\text{Sy.}}{1\tfrac{1}{2}} . 1\tfrac{1}{2} . 1 . 1 . \underset{\text{Ch.}}{53\tfrac{1}{2}} . 20 . 1 . \underset{\text{Sy.}}{\overset{15''}{16} . \overset{15''}{4}} . 4 . 2 . 1 . \underset{\text{Ch. } c=7}{\overset{15''}{39} . \overset{15''}{30}} . 6 . 1$

$\underset{\text{Sy. } c=15}{\overset{15''}{6} . \overset{15''}{2}} . 3 . \underset{\text{Sy. } c=7}{2}$

(The rest of this experiment is given on p. 305.)

EXPERIMENT III. (*a*).

Jan. 5, 1888. Large Dog. Secondary coil at 7 c.m., this gives shocks fairly strong to the tongue

Rise of saliva taken every 30 secs.

(1) 1.10. $1 . \underset{\text{Sy.}}{2\tfrac{1}{2} . 2\tfrac{1}{2}} . \tfrac{3}{4} . \tfrac{3}{4} . \underset{\text{Sy.}}{3\tfrac{1}{2}} . 2 . 1 . \underset{\text{Sy.}}{3} . 2\tfrac{1}{2} . \tfrac{1}{2} . \underset{\text{Ch.}}{82 . 45} . 3 . 2 . 1 . \tfrac{1}{2}$

$\underset{\text{Sy.}}{17\tfrac{1}{2}} . 4 . \underset{\text{Sy.}}{6} . 3 . \underset{\text{Sy.}}{4\tfrac{1}{2}} . 3 . 1\tfrac{1}{2}$

(2) 1.30. Chorda stimulated for a minute and a half. In a minute or two after end of stimulation, secretion stops.

2.36. $0 . 0 . \underset{\text{Sy.}}{6\tfrac{1}{2}} . 4 . 2 . 2 . 1\tfrac{1}{2} . 0 . \underset{\text{Sy.}}{3\tfrac{1}{2}} . 4 . 4$

(The rest of this experiment is given on p. 311.)

EXPERIMENT IV. (*a*).

Feb. 18, 1888. Dog. Weight 18 kilos. Chordo-lingual nerve uncut.

On cutting open the sheath of the vago-sympathetic nerve, the sympathetic was less distinctly visible than usual, in consequence the sympathetic as separated out contained some nerve fibres of the vagus.

$c = 7$, this gives shocks of moderate strength to tongue.

(1) **12.15 to 1.3. Stimulate chorda at intervals, collect 18 c.c. of saliva.**
1.27. Stimulate chorda 1 min.

15″ 15″ 15″ 15″ 60″ 60″ 15″ 15″

1.33. 0 . 13 . 7 . 7 . 5 . 14 . 1 . 3 . 7 . 9 . 5½ . 2½ . 1½ . ½ . 0 . 0 . 0 . 0

Sy. Sy vagus

15″ each 15″ each

0 . 5 . 9 . 9 . 6 . 5 . 1½ . ½ . 7 . 3 . 4 . 2 . 2 . 2 . 4½ . 3½ . 2½ . 1½

Sy. Sy. Sy.

2½ . 4 . 4½ . 3 . 2 . ½ . 0

60″ each 15″ 15″

(2) **1.54.** 235 . 225* . 55* . 18 . 5 . 3 . 2 . 2 . 1½ . 1 . 1 . 0 . 23 . 8 . 10

Ch. Sy.

15″ each

9 . 4 . 1½ . 0 . 0 . 5 . 4 . 4 . 4 . 6 . 4 . 4 . 3 . 1 . 1 . ½ . 0

Sy.

2.15. Cut chordo-lingual nerves.

7″ 8″ 15″

(3) **2.16.** 200 . 155* . 105* . 25 . 2 . 2 . 0 . 0 . 0 . 21 . 8 . 5 . 6 . 4 . 3 . 2 . 2

Ch. Sy.

½ . 0 . 0 . 0 . 0 . 7 . 4 . 4 . 3 . 3 . 2 . 2 . ½ . 0

Sy.

* The rise of saliva was rather greater than this, since the tube was of necessity emptied during the secretion.

(The rest of this experiment is given on p. 306.)

In these experiments it is seen that stimulation of the chorda tympani increases, without exception, the amount of saliva obtained by quickly following sympathetic stimulation. The secretion so obtained I shall sometimes for the sake of brevity call the 'augmented' sympathetic secretion. Fairly typical examples are those in Exp. I. (1) viz.

EXTRACT A.

Time during which saliva secreted	30 secs.	30 secs.	30 secs.	60 secs.	30 secs.	30 secs.	30 secs.	30 secs.	2 min.	8 min.
Saliva secreted measured in mm. of tubing	0	1½	1½	124	4	0	15	4½	3½	0
Nerve stimulated		symp.	symp.	chorda			symp.	symp.		

and in Exp. II. (3), viz.

EXTRACT B.

Time during which saliva secreted in seconds	30	30	30	30	30	30	30	15	15	30	30	30
Saliva secreted measured in mm. of tubing	1½	1½	1	1	53½	20	1	16	4	4	2	1
Nerve stimulated	symp.				chorda			symp.	symp.			

In the former of these, the chorda tympani was intact, in the latter, it was cut; this has little or no influence on the course of events, except when a reflex secretion is caused—a point which will be considered later.

If the sympathetic is stimulated several times in succession at short intervals after a chorda stimulation, the amount of saliva obtained from the stimulation of the sympathetic rapidly decreases.

Thus in Exp. III. (1)

EXTRACT C.

Saliva secreted in successive periods of 30″	1	3	2½	½	82	45	3	2	1	½	17½	4	6	3	4½	3	1½
Nerve stimulated		sy.			ch.	ch.					sy.		sy.		sy.		

Amongst other illustrations of this may be noted that in Exp. VI. (2) p. 304.

The rate of decrease varies somewhat in the different experiments, and in cases in which there is a prolonged after-secretion it may not be quite regular in the course of a single experiment (cp. Exp. IV.). The cause of the prolonged after-secretion I shall consider later.

The longer the first stimulation of the sympathetic is, and, within certain limits, the stronger the stimulus applied to it, the less is the subsequent sympathetic secretion above normal; thus a stimulation of the sympathetic for two minutes after a chorda stimulation may reduce the amount of saliva obtained by subsequent sympathetic stimulation to the amount obtained previous to the chorda stimulation.

The augmenting effect of chorda stimulation upon the sympathetic secretion disappears in time although the sympathetic is in the interval not stimulated. The time required

to do this seems to vary considerably. I have not made very many experiments on this point. The greater part of the effect certainly disappears in 10 to 15 minutes; for what length of time it may still be distinctly perceptible I am unable to say; in Exp. III. No. 2 there is apparently a case in which some effect remained after a pause of an hour, but other causes might have produced the result.

When a given stimulus gives rise in a tissue to greater functional activity than normal, the irritability of the tissue is said to be increased; so we may then put the conclusions given above in the following form:—stimulation of the chorda tympani increases the irritability of the gland for impulses reaching it by the sympathetic nerve; the increase in irritability slowly falls, and the fall is hastened by prolonging the stimulation of the sympathetic.

In the cases in the above experiments, in which the amount of the sympathetic secretion is taken during every 15 seconds instead of during every 30 seconds, it will be observed that the increase in its amount obtained by previous chorda stimulation is obtained chiefly although by no means entirely in the first 15 seconds of sympathetic stimulation. (Cp. Extract B, p. 297.) Further in all, or nearly all cases, the rate of secretion becomes very distinctly slower in the latter part of the period of 15 seconds. Thus in Exp. IV. (*a*), No. 3, in stimulating the sympathetic for 30 seconds, the saliva rises 21 mm. in the first 7 seconds, 8 mm. in the succeeding 8 seconds, and 5 mm. in the succeeding 15 seconds. And by carefully noting the rate of secretion it can be seen that it attains its maximum within two or three seconds or even in less than this, of its time of starting. The maximum is maintained for a variable time, there is then a steady fall, at first rapid and then slower and slower. The curve of the rate of secretion differs then, both from that of the chorda saliva and of ordinary sympathetic saliva.

The *duration of the chorda stimulation* affects the amount of saliva ensuing, on quickly following sympathetic stimulation comparatively little. I have made a few experiments only, with regard to this, but it appears to me that the maximum is soon attained, e.g. with a stimulation of a half-a-minute to a minute, if the stimulus is fairly strong. It is clear at any rate that a very brief stimulus of the chorda, stopped almost as soon as a secretion is observable, causes a very marked increase in the subsequent sympathetic saliva. The most notable case of this is the following from Exp. I. (No. 3).

EXTRACT D.

Saliva flow every 30 secs.	0	5	1	0	15	1	0	0	0	1½	½
Nerve stimulated		Chorda for 2 to 3 secs.			symp.					symp.	

Stimulate the chorda for 2 min. twice; before the after-secretion has stopped take the flow of saliva.

Saliva flow every 30 secs.	8	7	15	4	2½
Nerve stimulated			Sy.		

So far as prolonged stimulation of the chorda has a different effect from brief stimulation, it appears to increase the amount of the sympathetic saliva during its later rather than during its early period.

In the course of an experiment in which the chorda and sympathetic are repeatedly stimulated, the 'augmented' secretion very greatly diminishes; this seems to be chiefly due to the repeated stimulation of the sympathetic. The maximum amount which I have observed to be secreted in 15 seconds is a third of a cubic centimetre.

The saliva obtained is thick, viscid saliva, although less viscid than ordinary sympathetic saliva.

In the cat, so far as I have observed, the effect of stimulating the chorda tympani upon the subsequent sympathetic saliva is less than it is in the dog, and moreover, in the course of a protracted experiment, it may become inconstant.

Latent period. In making observations on the latent period I obtained tracings with the aid of Ludwig's kymograph, and of time-markers of the moment of stimulation and of the moment at which a rise of secretion could be observed in a tube of 2 mm. diameter lying over a graduated scale and connected with the cannula tied in the duct.

It is possible that the very beginning of the secretion, if it were slow, might thus go undetected. But by this method the results have been uniform, the 'augmented' sympathetic secretion has a less latent period than the ordinary sympathetic secretion. When the sympathetic is stimulated several times in succession, after a chorda stimulation, the latent period increases, as the amount of saliva

decreases. In any one animal the more copious the secretion the less the latent period. This latter result was obtained without exception in the few cases in which I took time tracings of the latent period and of the amount of secretion, but on account of the fewness of the cases, I am not prepared to say that exceptions may not occur.

In making the comparison between the latent period of 'augmented' and ordinary sympathetic saliva, the stimulus in the two cases must of course be the same. If the chorda stimulus be weak, though still producing a fairly copious secretion, the latent period of the immediately following sympathetic secretion, produced with a fairly strong current, may even be less than that of the chorda secretion.

Whilst speaking of the latent period I may mention one or two points about it, although probably they are well known. With a very weak chorda secretion, the latent period may be two to four seconds, although when the secretion occurs it is not a scanty one. After a certain (not too large) dose of atropin, stimulation of the chorda may fail to produce a secretion for half-a-minute or even longer (cp. Exp. IV. (*b*), No. 1, p. 306). After repeated stimulation of the sympathetic, it may sometimes happen that no secretion can be seen to take place for half-a-minute to a minute after the beginning of stimulation, occasionally only beginning when the stimulation of the nerve has ceased; in such cases I have never observed the secretion to be otherwise than scanty.

A copious secretion after a long latent period I have only observed when the chorda tympani nerve was intact, and the vago-sympathetic stimulated; that is to say when the copious secretion was a reflex one. An instance of this is given in Exp. I., No. 1.

EXTRACT E.

Time of flow of saliva in secs.	30	30	60	60	30
Flow of saliva in mm.	0	1½	8	15½	0
Nerve stimulated		vago-sympathetic			

In this case the secretion began at the end of the period of stimulation, synchronously with slight movements of the animal. According to Buff[1], stimulation of the vagus (and of other sensory nerves) only causes

[1] Buff, *Eckhard's Beiträge*, Bd. XII. p. 1, 1888.

a reflex secretion when it also causes some movements, and even then the secretion may be absent, especially after a large dose of the anaesthetic employed. It seemed to Buff that the latent period of the saliva obtained by stimulating the vagus was greater than that of saliva obtaining by stimulating other sensory nerves, e.g. the sciatic. He does not however give any time-data. The long duration of the latent period in the experiment I have quoted above, was no doubt due to the anaesthetic. As a rule I have obtained no reflex secretion from stimulating the vagus, the animals being deeply narcotized; in the instances in which it has occurred it has usually dragged on for several minutes. In this respect a difference in the effect of stimulating the vago-sympathetic and the sympathetic is indicated in Exp. I., No. 5.

Now and then it happens in an experiment that the flow of saliva produced by stimulating either the chorda or the sympathetic continues from two to ten minutes after the cessation of the electrical current. This I think is due to nervous stimuli continuing to pass down the nerve, and not to a spontaneous continuation of secretion by the gland cells. When this after-secretion occurs from stimulation of the sympathetic, the decrease in the amount of saliva in successive sympathetic stimulations is less than normal; if the first stimulation is made without the chorda having been stimulated a short time previously, and it produces an after-secretion, the second stimulation may produce a greater flow of saliva than the first; apparently a weak stimulation of the sympathetic increases the irritability of the gland. I propose making further experiments on the point.

Although the effect of stimulating either chorda tympani or sympathetic decreases somewhat in the course of a long experiment, in part no doubt in consequence of the exposure of the nerve, yet at times one or both nerves pass into a state, in which treatment, ordinarily without effect, causes a secretion. Thus simply taking up the nerve by its ligature and laying it across the electrodes preparatory to stimulating it, there being no electric current, may produce a flow of saliva. An example of mechanical stimulation, ordinarily ineffective, producing a flow of saliva is given in the following experiment, which shows some other points regarding secretion.

EXPERIMENT V.

Aug. 23, 1888. Dog. Weight 5¼ kilos. Chorda placed on electrodes or covered up but without any special precaution to prevent slight shifting by

occasional swallowing movements. Different coils were used for the chorda and the sympathetic.

12.38. Stimulate chorda.

(1) 12.51. Saliva flow each 30 secs. in mm.

0 . 6 . 5 . 3 . 3 . 0 . 0 . 1 . 11 . 1 . 7 . 4 . 3 . 2 . 1 . 1 . 0 . 6
(Sy. $c=8$; Pulled Sy. slightly; Sy.; Sy. for 5″, $c=7$)

0 . 4 . 0 . 2 . 1 . 0′ . 2 . 2 . 2 . 1 . 1 . 0 . 0 . ½
(Sy. for 5″; Sy. for 2″; Sy. $c=8$; Sy. for 5″)

(2) 2.17. Ch. $c=8$: 72 . 10 . 1 . 0 . – . 0 . 0 . 0 . 0 . 17 . 8 . Ch.: 37 . 13 . 5 . 0 . 0
(Sy. $c=8$)

Ch.: 15 . 63 . 17 . 11 . 7 . 4 . 1 . 12 . 1 . 1 . 0 . 0 . – . 0 . Ch.: 134
(Sy.; Dissect Sy. higher up)

16 . – . 0 . 19 . 5 . – . – . 2 . 1 . 6 . 4 . Ch.: 45 . 14 . 4 . Ch. $c=15$: 7 . 5 . 3
(Sy.; Sy.)

3 . Ch.: 19 . 8 . 2 . 2 . Sy.: 11 . 5

(3) 2.50. Shift chorda slightly on electrodes.

0 . Ch. $c=15$: 16 . 3 . 20 . 6 . Ch.: 11 . 2 . 0 . Ch.: 18 . 3 . 14 . 6 . 4 . 1
(Sy. $c=8$; Sy.)

Ch.: 6 . 4 . 1 . 0 . Ch.: 8 . 2 . 1 . 0 . Ch.: 11 . 2 . 3 . 0

(4) 3.15. 0 . 10 . 6 . 4 . 5 . 3 . 0 . 6 . 5 . 2 . Inject 330 c.c. NaCl ·6 into vein: 0 . 0 . 0 . 0 . 0 . ½ . 0 . ½ . ½ . 0
(Sy.; Sy.; Sy.)

½ . 2 . 3 . 2 . Inject 330 c.c. NaCl ·6: 6 . 2 . 1 . 5 . 7 . 5 . 5 . 1 . 1 . 0 . Inject 330 c.c. NaCl ·6: 0 . 5 . 10 . 6 . 2 . 1
(Sy.; Sy.; Sy.)

1 . 0 . 4 . 4 . 5 . 5 . 5 . 5 . 2 . 3 . 4 . 0 . 1 . 1 . 0 . 1 . 3 . 3 . 5 . 1 . 1
(Sy.; Sy.; Sy. $c=7$; Sy.; Sy.; Sy.; Sy.)

Ch.c=15 Ch.c=8 Ch.c=15 Ch.c=8

0 . 0 . 0 . 88 . 26 . 2 . 1 . – . 0 . 0 . 1 . 126 . 14

Ch.c=12

3 . 3 . 17 . 6 . 2 . 2 . 3 . – . 84 . 13 . 17 . 3 . 3

Sy.c=7 Sy.c=15 Sy.c=12

Fletcher and myself[1] found that the secretion obtained by pilocarpin or by stimulating the chorda tympani with fairly strong shocks, was increased by injecting into a vein a sufficient amount of dilute salt solution. In the experiment just given there was a slight increase in the sympathetic secretion during the injection, but the effect was apparently transient.

The injection did not here increase the effect of minimal stimulation of the chorda tympani, on the contrary shocks producing a secretion before injection failed to do so afterwards. This may have been due to a decrease in irritability of the gland in the interval owing to the lessened blood supply brought about by repeated stimulation of the sympathetic, or to other causes.

Comparison of rate of blood flow and rate of secretion when the sympathetic nerve is stimulated after the chorda tympani.

It has been shown by v. Frey[2] that when the sympathetic is stimulated with maximal shocks, the blood vessels of the sub-maxillary gland contract even if the chorda tympani be stimulated at the same time.

Since in my experiments maximal stimulation of the sympathetic after the chorda tympani gave a greater secretion than normal, it followed that the increased secretion could not be due to a delay in the contraction of the blood vessels. Still it seemed advisable to make matters quite clear by observing the blood flow at the same time as the secretion.

EXPERIMENT VI.

Aug. 20, 1888. Dog. Weight 7¾ kilos. All branches of jugular tied except the branches from the sub-maxillary gland. Inject into opposite jugular 22 c.c. of 10 p. c. peptone solution; this was commercial peptone

[1] *Phil. Trans. Roy. Soc.* Vol. 180, 1889 B, p. 109.

[2] v. Frey, *Arbeiten a. d. physiol. Anstalt zu Leipzig*, Jahrg. XI. p. 89, 1877.

consisting chiefly of albumoses. Separate battery and coil used in stimulating chorda and sympathetic. Chorda first gives a secretion with $c = 15{\cdot}5$; after this with $c = 16$. Stimulate sympathetic $c = 12$, movement of eye and dilatation of pupil, but no secretion; with $c = 10$, secretion.

Tie cannula in jugular and collect blood in tubes graduated in c.c.

(1) 5.0. Blood flow 9 c.c. in 4 min. $= 1\frac{1}{8}$ c.c. in 30 secs.

5.5. Blood flow and saliva noted every 30 secs. Chorda, $c = 14$; sympathetic, $c = 8$.

Blood flow in c.c.	1	1	$1\frac{1}{4}$	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Saliva flow in mm.	0	0	0	5	4	4	4	6	5	4	3	9			
				Sy.				Sy.				Sy.			

$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$
5	2	–

(2) 5.14.

Blood flow	$\frac{1}{2}$	$1\frac{1}{4}$	2	1	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{3}{4}$	1	1	1	$\frac{3}{4}$	$\frac{1}{2}$ –													
Saliva	–	85	25	3	25	6	3	2	1	$\frac{1}{2}$	$\frac{1}{2}$	0	7	3													
		Ch.			Sy.								Sy.														

$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{3}$	1	$1\frac{3}{4}$	–	1	1	1	$\frac{1}{2}$ –	$\frac{1}{3}$	$\frac{1}{5}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{1}{2}$ –	$\frac{1}{4}$										
5	5	3	3	40	33	7	4	3	3	63	9	4	–	–	–	11	4										
				Ch.						Sy.						Sy.											

$\frac{1}{2}$ –	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$ –	
2	5	2	3	1	
	Sy.		Sy.		

Blood has formed a loose clot in the cannula.

(3) In the following the chorda was stimulated $c = 10$ and Sy. $c = 7$.

																15″	15″	15″	15″								
5.45. Blood flow	1	$2\frac{1}{4}$	$2\frac{1}{2}$	$3\frac{1}{4}$	$\frac{3}{4}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$ –	$\frac{1}{5}$	$\frac{1}{4}$																	
Saliva	54	23	28	36	63	29	9	4																			
	Ch.				Sy.																						

(4) Inject 10 c.c., 10 p.c. peptone.

5.54. Blood flow	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{4}$	$\frac{1}{2}$	$1\frac{1}{3}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	1	$\frac{1}{4}$	$\frac{1}{2}$ +	$\frac{1}{2}$ –													
Saliva	4	4	4	8	3	37	10	12	15	19	23	8	6	5													
				Sy.		Ch.					Sy.		Sy.														

Inject 50 mgrms. atropin sulphate—the chorda then gives even a less increase of blood flow than in the preceding observation. Stimulation of the sympathetic after the chorda gives a flow of saliva varying from 1 to 3 mm. in 30 secs.

The blood at no time during the experiment issued from the gland of an arterial tint, on stimulation of the chorda.

In the first part of the experiment, a weak stimulation of the sympathetic produces a diminution in the blood flow whether it is, or is not, preceded by a weak stimulation of the chorda; but the amount of saliva in the two cases is very markedly different. Stronger stimulation of the sympathetic decreases to a much greater extent the blood flow, but still gives the augmented secretion after stimulation of the chorda. Hence, the augmented sympathetic secretion is not due to a failure on the part of the sympathetic to cause the normal constriction of the blood vessels.

Effect of atropin given in sufficient quantity to paralyse the secretory fibres of the chorda tympani.

The effect of atropin upon the power of the chorda tympani to increase the subsequently secreted sympathetic saliva depends in any one experiment upon the amount of atropin injected. The amount of atropin required to produce a given effect may however be very different in different experiments. We may distinguish two stages in the action of atropin; one in which the power of the chorda to produce a secretion is very nearly abolished, but its power to increase the subsequent sympathetic secretion is still considerable, and another in which its secretory power is abolished and its power over the sympathetic secretion is reduced to very narrow limits. It is true that the former stage I have only observed twice, but there is reason for thinking that when it was not observed too much atropin was given at the first injection. We will consider the latter stage first. An instance in which the gland was in this stage or bordering on it I have given in a previous paper[1]. Fifteen mgrms. of atropin were in this case injected into the jugular vein of a large dog; after this, stimulation of the chorda tympani had but a slight effect upon the sympathetic secretion, increasing its rate of flow about three times.

EXPERIMENT II. (*b*).

Jan. 2, 1888. Dog. Weight 6¼ kilos. For the beginning of the experiment see p. 295.

1.30. Inject into jugular vein on the opposite side of the body to that on which flow of saliva is being observed 1 c.c. of 1 p. c. atropin sulphate diluted with 4 c.c. sodium chloride ·6 p. c., i.e. 10 mgrms. atropin sulphate were injected.

[1] This *Journal*, Vol. IX. p. 58, 1888.

(1) 1.32. Flow of saliva taken each minute:

0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 1 . ½ . ½ . 0 . ½ . ½ . 0 . 1½
Ch. (c=8) Sy. Ch. Sy. Ch. Sy. Sy. (c=7) Ch. Sy.

½ . ¼ . ¼ —saliva now taken each 30 secs. 2½ . 1½ . 1 . 1 . ¼
Sy. Ch.

2¾ . 2 —pause, rise of 2½ mm. in 15½ min.
Sy.

(2) 2.22. 0 . ½ . 0 . 0 . 1 . ½ . 1 . ½ . ½ . ½ . ½ . ½ . ½ . ½ . 1 . ½ . ½ . 2 . 2 . 1
Sy. Sy. Sy. Ch. Sy.

1 . 1½ . 2 . ½ . ½ . 1 . ¼ . ¾ . ½ . 1 . ½ . ½ . 3 . 1½ . 4½ . 1½ . 1
Sy. Sy. Sy. Ch. Sy.

1 . 2 . 1½ . 2½ . 2 . 1½ . 1½ . 2 . 2 rise of 2½ mm. in 3 min.
Sy. Ch. Sy.

(3) 3.3. ½ . 0 . 0 . 4½ . 1½ . 2 . 2½ . 1 . 3 . 7 . 1½ . 1 . 2 . 2½ . 1½ . 1½ . 3
(15″ 15″)
Sy. Ch. Sy. Ch. Sy. Sy. (c=5)

Here, after 10 mgrms. of atropin, stimulation of the chorda has very little effect upon the amount of the sympathetic saliva. At first sight it appears to have no effect, but this point we will consider later. A similar action of atropin is shown after so small a dose as 5 mgrms. in Exp. X. and Exp. XII. (*b*). Other examples are given in Exp. VI.

Hence, a sufficient—not necessarily a large—dose of atropin reduces to very narrow limits the power of the chorda tympani to increase the subsequent sympathetic saliva.

The amount of atropin given in the above experiment does not, as is well known, abolish the vaso-dilator power of the chorda tympani. And this is seen in the following experiments.

EXPERIMENT IV. (*b*).

Feb. 18, 1888. Dog. Weight 18 kilos. For the beginning of the experiment see p. 295.

Rise of saliva taken every 30 secs.

(1) 2.37. 150 . 80 . 23 . 3 . 2½ . 0 . 0 . 0 . 5½ . 4 . 4 . 3 . 3 . 2 . 1½
Ch. Atropin* Ch.

½ . 0 . 0 . 0 . 0 . 1 . 1 . 4 . 4 . 0 . 17 . 2 . 6½ . 6½ . 3 . 1 . 0
Ch. Sy. for 15″ Sy.

* Inject 1·5 c.c. of ·5 p. c. atropin—i.e. 7·5 mgs. into jugular vein.

No further secretion in 6 min. Stimulate chorda: no secretion.

(2) 3.7. Stimulate chorda $c = 5$: no secretion.

15″ each — 15″ each

3.13. 0 . 1 . 4 . 2 . 2 . ½ . 0 . 0 . 0 . 0 . 0 . 0 . 0 . ½ . 1 . 2 . 2 . 1½ . ½ . 2

Sy. $c=7$ — Ch. $c=5$ — Sy. $c=7$

15″ each

1 . ½ . ¼ . 0 . 0 . 0 . 0 . 0 . 0 . 0 . ½ . 2 . 3 . 2 . 1½ . ½ . 0

Ch. $c=5$ — Sy. $c=7$

(3) 3.29. 0 . 1 . 1½ . ½ . 1 . 3 . 3½ . 1½ . 1 . 3 . 1 . 0 . 1 . 5½ . 3 . ½

Sy. — Sy. — Ch. — Sy.

Dissect out gland, find one largish gland vein opening into lower branch of jugular, ligature jugular on either side of this, tie in a cannula and collect blood in a tube graduated in cubic centimetres.

Flow taken each minute unless otherwise mentioned. At each vertical line there was a pause.

Rise of Saliva	0 . 0 . 0 . 0 . 0 . 0	0 . 0 . 0 . 2 . 8 . 1 . 3 . 7 . 1
Flow of Blood in ·5 c.c.	3 . 3 . 2½ . 2½ . 2½ . 7½ (Ch.)	2½ . 4½ (Ch.) . 3 . 1½ (Sy.) . 1½ . 2 . 1 (Sy.) . 2 . 3

30″ 15″ 15″ 30″ 30″

0 . 0 . 0 . 0 . ½ . ½ . 1 . ½ . ½ . 0	0 . 0 . 0 . 0 . 0
4 . 6 . 5 . ½ (Ch. / Sy.) . 2 . 2½ . 2½	2 . 8½ (Ch.) . 3½ . 3 (*) . 2½

0 . 0	0 . 0
2 . 2	2 . 2

30″ 30″

0 . 0 . 0 . 0 . 1½ . 2 . ½

3 . 5 . 7 . 1 (Ch. / Sy.) . 2½ . 2

* Inject into opposite jugular ·5 c.c. pilocarpin ·2 p.c.

EXPERIMENT XII. (*b*).

Aug. 21, 1888. Dog. For beginning of experiment see p. 326. 5 mgrms. of atropin were injected after pilocarpin.

Stimulation of the sympathetic gives a scanty secretion from the submaxillary gland, and this is not increased by previous stimulation of the chorda tympani. Arrange to measure blood flow from the gland.

4.23. Blood flow from gland in ½ c.c. every 30 secs.

1½ . 10 (Ch.) . 6½ . 3 . ⅛ . ⅛ . ⅛ (Sy.) . ¼ . 1 . 1 . 1

4.30. Blood flow in ·5 c.c.	2	9	9½	3½	1	½	½	–	
Saliva in mm.	0	0	0	0	1	3	2	0	
			Ch.		Sy.				

In both of these experiments the blood flow is considerably increased by stimulating the chorda tympani; in the latter the rate of blood flow is increased five times, yet this causes little or even no increase in the amount of the sympathetic saliva secreted immediately afterwards. Hence the increase of sympathetic saliva which normally follows stimulation of the chorda tympani cannot—except in small part—be due to the action of its vaso-dilator fibres, but must be due to the action of its secretory fibres.

Bradford[1] in a recent paper comes to a similar conclusion with regard to the 'occasional sympathetic saliva seen after previous stimulation of the cerebral nerve;' it is he thinks 'probably due to changes in the gland cells or gland nerve cells produced by the antecedent stimulation.' In connection with this he says that I explained[2] the parotid secretion which I obtained in the dog after stimulation of Jacobson's nerve as being the result of vascular dilatation. This is an oversight on his part. I expressly stated that I reserved the working out and the discussion of the effects of blood flow on both the sub-maxillary and on the parotid gland for a later and fuller communication. I knew in fact from the action of atropin that a part of the increase in the effect of stimulating the sympathetic, produced by previous stimulation of the cerebral nerve, was due to the secretory and not to the vaso-dilator fibres of the latter. And in the following month I published[3] an account of an experiment in which a dose of atropin, which has very little influence on the vaso-dilator fibres of the chorda tympani, deprived the chorda tympani of much the greater part of its influence on the secretion of sympathetic saliva.

A point which is not shown in the experiments given, but which I have observed in all the cases tried, is that atropin does not abolish the increased irritability of the gland which is produced by stimulating the chorda tympani before the injection of atropin; thus if the chorda tympani be stimulated and then atropin given in sufficient dose to completely paralyse the secretory fibres of the chorda, stimulation of the sympathetic produces a fairly plentiful flow of

[1] This *Journal*, Vol. IX. p. 287, October 1888.
[2] *Proc. Physiol. Soc.*, February 1888.
[3] This *Journal*, Vol. IX. p. 58, March 1888.

saliva as if atropin had not been given. On repeating the stimulation of the sympathetic two or three times, the amount of saliva obtained rapidly falls, and it cannot then in any way be notably increased (cp. also p. 324).

We may here say a word or two about the effect which variations of blood flow have upon the secretion of the salivary glands.

(*a*) When the secretory fibres are being stimulated, an increase of blood flow up to a certain limit increases the rate of secretion. This has been shown by Heidenhain[1] in the case of the chorda tympani.

When sufficient pilocarpin is injected into the vein of an animal, a stage is obtained, as I have shown[2], in which further injection of pilocarpin causes little or no increase in the rate of secretion. In this stage the secretion is slow, and is only slightly increased by stimulating the chorda tympani. I take the secretory fibres of the chorda to be stimulated to or nearly to their maximum extent, and the slight increase which occurs on stimulating the chorda tympani to be chiefly due to the increased blood flow[3].

Similarly I should explain many instances of increased secretion which are obtained on stimulating the chorda tympani after the sympathetic in an atropinised animal. The sympathetic secretion may have become very slow, but start again as the blood flow, owing to stimulation of the chorda, increases (cp. Exp. II. (*b*), No. 2, p. 306.)

(*b*) A considerable decrease of blood flow decreases, in proportion to its continuance, the irritability of the gland, when the blood flow becomes normal the irritability returns to normal in a time depending on the duration of the previous decrease in the blood flow; the return to

[1] *Studien des physiol. Instituts zu Breslau*, Hft. IV. 1868, p. 96.

[2] This *Journal*, Vol. I. p. 366, 1878.

[3] Gley (*Archives de physiol. norm. et path.* 1889, p. 151) in the course of an account on some observations on secretion, states that when 5 to 10 mgrms. of pilocarpin are injected into the pleural cavity of a dog, stimulation of the chorda tympani will cause little or no increase in the secretion as long as it is abundant. He quotes me as saying (*Journal of Anatomy and Physiology*, Vol. IX. 1876) that stimulation of the chorda causes an increase in all stages of the pilocarpin secretion. The quotation is perfectly correct as far as it goes, but my statement was only for the case of an amount of pilocarpin not much removed from minimal; I pointed out that by repeating the injection several times a stage was reached in which stimulation of the chorda had a very slight effect on the saliva. Somewhat later in returning to the subject (this *Journal*, Vol. I. 1878, p. 339) I gave several instances in the cat, in which stimulation of the chorda produced little or no effect, both during the rapid pilocarpin secretion at the beginning of an experiment with a small amount of pilocarpin, and during a slow pilocarpin secretion at the end of an experiment with a larger amount of pilocarpin.

normal irritability is quicker, if the blood flow be increased above normal.

Heidenhain[1] has shown that the irritability of the sub-maxillary gland, i.e. either of its nerves or of its cells, is injured for a considerable time by stopping or by greatly reducing, for about five minutes, the flow of blood through it. Since stimulation of the sympathetic largely reduces the blood flow through the gland, protracted or repeated stimulation of it should produce an effect like though less than that produced by compressing the arteries in Heidenhain's experiments.

We have seen (p. 297) that the increased irritability brought about by stimulating the chorda tympani is soon abolished by stimulation of the sympathetic. Again, when a secretion is produced by pilocarpin, stimulation of the sympathetic, if not too brief, leaves an after-action such that the secretion only slowly regains its previous rate. The time required for the secretion to regain its rate is apparently longer than that required for the vaso-constrictor effects to pass off. Some instances of this I have given in an earlier Paper on the effect of pilocarpin on the salivary secretion in the cat. A more striking instance in the dog is given below (p. 326).

It is natural to suppose that a decrease in irritability brought about by a lack of blood should disappear the more quickly the more copious the subsequent supply. And I attribute the effect of stimulating the chorda tympani in Exp. II. (*b*) in the main to a restorative effect of the greater flow of blood thereby caused. The increase in the sympathetic secretion obtained in this manner differs from the 'augmented' sympathetic secretion, in that the increase is not chiefly at the beginning, but is either fairly equally distributed throughout the whole period of secretion, or even affects least the earliest period.

It deserves mention that according to Bradford a single stimulation of the sympathetic, even when producing no secretion, considerably diminishes the amount of saliva obtained by the subsequent chorda stimulation. My experiments on this point have been confined to the cases in which stimulation of the sympathetic produces a secretion. I find that sympathetic stimulation may or may not decrease the amount of saliva obtained by quickly following chorda stimulation. The result depends upon the strength and duration of both stimuli. The depressing action when it occurs soon disappears. Some of the results

[1] *Op. cit.* p. 96.

are curious. I propose giving an account of them in a later Paper. I am unable to agree with Bradford in thinking that the sympathetic produces any special action upon the gland in consequence of which the chorda saliva is decreased. Whatever action it may have in this direction—after from exhaustion accompanying secretion—I attribute to the action of its vaso-motor fibres[1], since with properly adjusted stimuli, simultaneous stimulation of the chorda and the sympathetic gives a more plentiful secretion than does stimulation of the chorda alone. Details with regard to this I shall give in a later Paper.

Effect of atropin when given in quantity not quite sufficient to paralyse the secretory fibres of the chorda tympani.

I have spoken above of a preliminary stage in the action of atropin in which the secretory power of the chorda tympani is reduced to a much greater degree than is its augmenting power on sympathetic saliva. This I have only observed twice.

EXPERIMENT III. (*b*).

Jan. 5, 1888. Large dog. For the beginning of the experiment see p. 295.

2.44. Inject into branch of jugular 1 c.c. of ·5 p.c. atropin sulphate. The atropin solution was not freshly made and was probably of less than its nominal value.

(1) 2.46. Rise of saliva every 30 secs. unless otherwise mentioned.

60″ 60″ 60″
1½ . 4 . 3 . 3 . 2 . 1 . ½ . 1 . 4 . 3 . 1½ . 1½ . 1 . 1 . 1 . 16
Ch. (*c*=7) Ch. Sy.

4 . 1 . ½ . ½ . ½ . 0 . 0 . 0 . 0 . 14 . 4 . 3 . 6 . 3 . 5 . 2½ . ½ . 2
Inject 1 c.c. ·5 p.c. atropin Ch. Sy. Sy. Sy. Ch.

11½ . 1 . ½ . 2½ . 3 . 1½ . 1½ . 1 . 1 . 1 . 1 . ½ . 1 . ½ . 1 . 0 . 18
Sy. Ch. Clamp trachea tubes Ch. Sy.

[1] Apropos of this Bradford quotes me as saying (This *Journal*, Vol. I. 1878, p. 101) that in the cat simultaneous stimulation of the chorda and of the sympathetic *always* causes a greater secretion than is obtained by stimulating the chorda alone. I stated this to be the case when the stimuli were not far removed from minimal; but stated also that when the stimuli are strong, simultaneous stimulation gives less secretion than that obtained from the chorda alone.

4 . 1 . ½ . 4 . 3½ . 1 . 0 . 1½ . 1½ . 3 . 3 . 2 . 2 . −1 . −1 . 3½ . 5½
Sy. Sy. Sy. Ch. Ch.

4 . 16 . 6 . 4 . −2
Sy.

3.26. Inject 1 c.c. ·5 p.c. atropin.

(2) 3.35. 0 . 1 . 0 . 1½ . 2 . 1½ . 2 . 0 . 0 . 1 . 1 . 1 . 3½ . ½ . 3 . ½ . 1½
Sy. Sy. Sy. Ch. Ch.

8 . 3 . 1 . 4
Sy. Sy.

A similar result was obtained at the end of Exp. VIII. (p. 321).

About 1·8 c.c. of atropin sulphate, nominally ·5 p.c. (the same solution as that used in Exp. III. (*b*)), were injected; the chorda stimulated for a minute gave no appreciable secretion, but considerably increased the amount of saliva obtainable by stimulating the sympathetic.

It will be seen from the above experiment that after a certain dose of atropin, stimulation of the chorda tympani although giving rise to little if any secretion of saliva yet considerably increases the amount of the subsequent sympathetic saliva, and may do so about as much as it does normally, i.e. when it produces a copious secretion. This is clearly shown in the following extract from Exp. III.

EXTRACT F.

Exp. III. (*a*). Before atropin.

Saliva flow every 30 secs.	½	82	45	3	2	1	½	17½	4	6	3	4½	3
Nerve stimulated		ch.	ch.					sy.		sy.		sy.	

Exp. III (*b*). After atropin.

Saliva flow every 30 secs.	½	1	½	1	0	18	4	1	½	4	3½
Nerve stimulated		chorda	chorda	chorda		symp.				symp.	

When I first observed this effect I was inclined to attribute it in great part to the increased blood flow through the gland increasing the

irritability of the gland. But a little later in writing an account of the action of atropin upon the chorda tympani[1], I had occasion to point out that it was not easy to determine the exact time of paralysis of the secretory fibres of the chorda, since, amongst other reasons, the chorda may give a slight secretion on the second or third stimulation, although it does not on the first stimulation. And this naturally suggested that the cases in which, after atropin, the chorda augmented the subsequent sympathetic saliva, though itself causing little or no secretion, the effect might be due to the remaining irritability of the secretory fibres rather than to the increase of blood flow. This I take to be the explanation. It does not, it is true, seem probable *à priori*, that a nerve which on maximum stimulation gives at most a very slight secretion should nevertheless be able to increase the irritability of the gland to such an extent that the amount of secretion obtained by stimulating another nerve is considerably increased. The following considerations however tell strongly in favour of this actually occurring. It is clear that at a certain stage of atropin poisoning, stimulation of the chorda, with whatever strength of shocks, causes but a slight stimulation to the gland; and it is possible that at a little further stage stimulation of the chorda may increase the irritability of the gland without actually producing a secretion. In some experiments made on the cat[2] I found not infrequently that stimulation of the chorda or of the sympathetic with minimal stimuli produced a greater secretion if the other nerve were at the same time stimulated with shocks which were a trifle less than the minimal shocks capable of producing a secretion, and this may fairly be interpreted as meaning that sub-minimal stimuli may increase the irritability of the gland. I found also in the few experiments tried on the point that a minimal stimulus increased the effect of a subsequent minimal stimulus. Bradford, apparently without knowing of the above, has made some observations of a similar nature in the dog; he has found that often at the beginning of an experiment a stimulus ineffective the first time, might become so on repeating it three or four times, and that when a secretion was obtained the stimulus might be made weaker and yet still cause a secretion. I have also often observed at the beginning of an experiment, or after a long pause, that the minimal effective stimulus for the chorda diminished somewhat as soon as a secretion was produced.

Another observation of Bradford's which bears on this point is,

[1] This *Journal*, Vol. IX. p. 55, 1888.
[2] This *Journal*, Vol. I. p. 103, 1878.

I think, that as a rule stimulation of the chorda tympani with shocks not exceeding five a second, or with sub-minimal more rapid shocks, causes little or no secretion but does cause a considerable electrical effect (second phase) in the gland.

All this goes to show that a stimulation of the chorda which produces no secretion, or a very slight one, may yet produce an appreciable effect upon the gland.

That the increased irritability of the gland is not due to the increased blood flow is also indicated by the fact that the additional amount of atropin sufficient to prevent the increase of irritability on stimulating the chorda tympani, is not sufficient to make any considerable difference in the increased amount of blood which will flow through the gland on chorda stimulation.

Effect of obstructing for some time the outflow of blood from the gland vein.

In the following experiment the effect of injuring the irritability of the gland by interfering with the outflow from the gland vein is shown.

EXPERIMENT VII.

Simultaneous stimulation of chorda and sympathetic. Saliva and blood flow.

Oct. 3, 1888. Dog. Weight 15 kilos. 4 c.c. 5 p.c. morphia injected subcutaneously. A large gland vein opens into the lower branch of the jugular, and a small one into the upper branch. Lower branch of jugular tied for about 20 minutes. Chorda and sympathetic placed on protected electrodes, and covered up. Inject into crural vein 40 c.c. 10 p.c. peptone. Cannula tied in lower branch of jugular (into which large vein of gland opens), branches of jugular except gland vein being ligatured; cannula connected with a burette graduated in c.c. Blood and saliva flow taken each 30 secs.; at times the flow could not be taken since one or other of the tubes required to be emptied, this is indicated below by 'empty.'

					Ch. $c=6{\cdot}5$					Ch.						Ch.
(1)	1.3.	Saliva flow in mm. of tubing	0	. 0	. 10	. 5	. 3	. 29	. 6	. 2	. 2	. 2	. 33			
		Blood flow in c.c.	·5	. ·6	. 3·1	. ·9	. ·1	. 1·3	. ·1	. ·4	. ·4	. ·4	. ·1			
							Sy. $c=7$						Sy. $c=6$			

		Ch.						Ch. $c=6$								Ch.		
	6	24	3	2	1	25	3	1	empty	1	12	2	1	0	1			
	·7	·2	·7	·3	·2	·1	·4	·1	·1	·2	1·6	·1	0	0	·1			
		Sy.				Sy.							Sy. $c=9$		Sy. $c=8$			

Ch.						Ch.											
8	1	4	1	4	24	9	9	8	7	7	3	1	2	0			
1	·1	0	0	0	0	0	0	0	0	0	·3	·3	·3	·1			
		Sy. $c=7$				Sy. $c=6·5$											

The blood in the burette had become very thick, offering great resistance to the flow of blood.

		Ch. $c=8$		Ch. $c=7$				Ch.								Ch.		
(2)	1.43.	0	5	1	6	1	15	20	15	2	1	0	0	25	72	11	11	
		·5	3	·7	1·8	·5	0	0	·8	empty	1	1	4·3	·2	·9	·9		
							Sy. $c=6·5$							Sy.				

						Ch.*		Ch.				Ch.					
4	empty	18	69	11	34	11	32	57	empty	5							
·2	·3	0	0	·4	1·9	·7	0	·5	·7	0							
		Sy. $c=7$					Sy. $c=8$										

Ch. $c=6$							Ch.	
122	100	20	empty	28	17	26	130	
2·6	1·8	·5	0	0	0	0	0	clot in cannula
	Sy. $c=9$			Sy.		Sy.		

* The chorda was here rearranged on the electrodes.

				Ch.		Ch.				Ch.							
(3)	2.4.	4	4	130	23	62	empty	5	32	98	15	14	6				
		1·4	1·5	5·1	1·2	3·3	1·9	·6	·1	2	·8	0	·1				
				Sy.					Sy.			Sy.					

(4) Inject 4 c.c. ·1 p.c. atropin sulphate (i.e. 4 mgrms.) into crural vein.

		Ch.						Ch.									
	2.15.	0	3	0	0	6	2	0	1	1	3						
		4·6	2·3	·6	·4	·1	0	·6	·9	·3	0						
			Sy.			Sy. $c=8$					Sy.						

Here there are two noteworthy results. In the first part of the experiment, the gland has not recovered from the injury previously inflicted by preventing the outflow of blood from the chief gland vein. In this state stimulation of the chorda produces very little secretion,

and further, although increasing considerably the blood flow, augments little if at all the sympathetic secretion. The same result was obtained in one other similar experiment tried. Hence then, when the irritability of the gland is greatly diminished by interference with the blood flow through it, stimulation of the chorda tympani may very slightly augment the subsequent sympathetic saliva.

A second result to notice is that simultaneous stimulation of the chorda and sympathetic produces a greater secretion than the sum of the secretions produced by separate stimulation. This is the more remarkable, since, as has just been pointed out, stimulation of the chorda does not increase the amount of saliva obtained by *subsequent* sympathetic stimulation. Putting the two results together it would appear, that in a certain state of greatly diminished gland irritability, stimulation of the chorda tympani increases the irritability of the gland during the stimulation for impulses reaching it by the sympathetic, but this increase rapidly disappears after the cessation of the stimulus. It will be noticed that in the various cases just mentioned, the saliva obtained is largely independent of the amount of blood flowing through the gland.

Another stage is reached later in the experiment, a stage like the above except that stimulation of the chorda augments the subsequent sympathetic saliva. The secretory power of the chorda and its augmenting power on the sympathetic saliva rapidly increase and the normal condition is soon obtained.

Note on the blood flow through the gland.

In the preceding experiments some of the variations which occur in the blood flow through the gland are given. These we may compare with the result obtained by v. Frey[1]. It is to be noticed that the normal blood flow through the gland was much less in my experiments than in v. Frey's, chiefly no doubt because of the amount of anaesthetics given. Further in my experiments albumoses were as a rule injected; this prevented the formation of a firm clot, although it did not always prevent, especially in the later stages of an experiment, the blood flow becoming very thick and even jelly-like; in a few cases only however did actual clotting in the vein occur even when the flow was slowest.

[1] *Ludwig's Arbeiten*, Jahrg. XI. 1876, p. 89.

The results agree in nearly all points with v. Frey's. There are a few which offer a slight variation from his or which were made in a somewhat different manner. These I may mention. v. Frey pointed out that a strong stimulation of the chorda might overcome a weak stimulation of the sympathetic. This occurs within much wider limits of sympathetic stimulation in my experiments than in v. Frey's. Thus a sympathetic stimulation which is able to reduce the blood flow so far that it nearly ceases, may have hardly any effect if the chorda is simultaneously stimulated, e.g. in Exp. VII (3).

EXTRACT G.

Stimulate		Ch.		Ch.				Ch.			
Blood flow each 30 secs. in c.c.	1·5	5·1	1·2	3·3	1·9	0·6	0·1	2·0	0·8	0	·1
Stimulate		Sy.	Sy.				Sy.	Sy.		Sy.	

Further v. Frey found that if the sympathetic be stimulated during the period of vascular dilatation caused by stimulating the chorda, the constrictor effects were apparent during the stimulation of the sympathetic only; this does not seem to me to be always the case, for instance in the following from Exp. XII. (*b*).

EXTRACT H.

Blood flow in each 30 secs. in ½ c.c.	1½	10	6½	3	⅛	⅛	⅛	¼	1
Nerve stimulated		chorda			symp.	symp.			

It is possible, though I do not think it is probable, that the apparent after-effect of stimulating the sympathetic in such cases may have been due to slight clotting taking place in the vein which was only gradually washed away.

There is some evidence also that when the chorda is stimulated soon after the sympathetic has been stimulated, it causes for 30 to 60 seconds a less increase of blood flow than it otherwise would, i.e. there is an after-action in the part of the sympathetic which diminishes for a time the effect of stimulating the chorda.

Note on some experiments by Bradford.

Bradford in a recent paper upon various points in the physiology of the salivary glands touches upon the question I have here dealt with. In doing so he apparently considers that the results described in my preliminary communication had their origin in and confirmed some previous results of his own[1]. In view of this I am obliged to say that neither the one nor the other is the case.

Bradford's results were as follows:—In two cases he obtained a copious secretion of saliva from the sub-maxillary gland of the dog on stimulation of the sympathetic; in one case he obtained a scanty secretion of one drop of saliva from the parotid gland on stimulating the sympathetic. These results, as he points out, had been noted by other observers. Bradford however discussed these results and as he did not observe them in the earlier part of the experiments but only after repeated stimulation of the cerebral nerve, he came to the conclusion that they were probably due to exhaustion either of the gland cells or of the peripheral nervous structures. He says "The effect is most readily obtained in an exhausted gland...In the case of the parotid also, it was only after repeated excitation of the tympanic plexus that stimulation of the sympathetic caused the scanty secretion described above; so that here too it is apparently a question of exhaustion." It is true that previous stimulation of the cerebral nerve is mentioned, but it is never mentioned as producing any direct effect, but only as leading on frequent repetition to exhaustion of the gland. Since the result was not always obtained I accepted it as one of the many points regarding salivary secretion which we only imperfectly understand.

Some time afterwards I found in the manner I have already mentioned, that sympathetic stimulation produced invariably a greater secretion than normal from the sub-maxillary gland for some time after stimulating the chorda tympani, and produced invariably a secretion from the parotid gland for some time after stimulating Jacobson's nerve. In writing out the preliminary account of this, I turned to Bradford's paper to compare the comparatively free secretion of sympathetic sub-maxillary saliva which I obtained with that which he had described. I found such differences between the two that I concluded that they were of different causation.

Bradford, as has been said, observed a copious secretion to follow stimulation of the sympathetic in two cases, in one of these the details

[1] This *Journal*, Vol. VIII. p. 92, 1887.

are given. This I may reproduce to allow of a comparison with the experiments given in this paper.

"In the first case the sympathetic was being stimulated, and was yielding as usual a scanty viscid secretion accompanied by a variation, indicating the surface of the gland to become positive to the hilus. This variation, as is usual, was a slow one and was rather small; suddenly the galvanometer indicated a large first phase and coincidently with this, a rapid and copious secretion of watery saliva occurred." At 11.15 the chorda was stimulated; then the experiment was as follows:

Time.	Nerve.	Saliva.	Electrical variation.		Coil.	
			sign	amount	strength	duration
11.20	sympathetic	1·2 drops viscid	+	50	80 mm.	10″
11.25	„	2 drops	+	80	50 mm.	10″
11.30	„	12 drops } long latent period	+ –	+ 80 – off scale	50 mm.	10″
11.35	„	14 drops } long latent period	+ –	+ 50 – off scale	50 mm.	10″

The paragraph quoted above implies that at 11.30 and at 11.35 the secretion started as a slow secretion causing the usual + electrical variation, and then rapid secretion began, producing a – electrical variation. It is not quite clear that this is meant, but if it is it constitutes an important difference between the rapid secretion here and the rapid sympathetic secretion which I describe, for this never begins with a slow secretion like the ordinary sympathetic saliva. Apart from this there are two differences to notice (1) that the first and second stimulations of the sympathetic produce a scanty secretion, and that the rapid secretion is produced by the third and fourth stimulations; (2) that the latent period is long 'much longer than the usual one.' I am not prepared to say that these differences may not be due to some differences in the manner of conducting the experiment. It is possible that his results have the same causation as mine, but as I have not actually observed a secretion, such as he describes, I cannot claim to confirm its occurrence.

The Parotid Gland.

Stimulation of the sympathetic has rarely been found to give rise to a secretion from the parotid gland and still more rarely to give rise

to a secretion exceeding a few drops. An account of the earlier experiments are given by Heidenhain[1]. Eckhard[2] in no case observed a secretion to follow stimulation of the sympathetic. Navrocki[3] obtained it twice out of numerous experiments, in one case a very scanty secretion was obtained, in the other a less scanty secretion. Heidenhain also observed twice out of many trials a scanty sympathetic secretion, in one case two drops of saliva, in the other three were slowly secreted.

Since it was possible that the scanty secretion obtained might be simply pressed out of the gland by the contraction of the surrounding muscles; it was generally assumed that the sympathetic contained no secretory fibres for the parotid, or at any rate very rarely contained them.

In experiments made at various times I seldom obtained a secretion; and in one case only[4], more than one to two drops. In this case, the cerebral secretory nerve was not cut, but the high percentage of solids in it—for enough was obtained for analysis—showed that it was not saliva such as is obtained on stimulating the cerebral nerve.

Bradford[5] observed in one case a scanty secretion (1 drop of saliva); this was after repeated stimulation of the tympanic plexus so that he was inclined to attribute the result to exhaustion (cp. above, p. 318).

In my preliminary account I stated that for a short time after stimulation of Jacobson's nerve, the sympathetic on stimulation produced without fail a secretion, and I gave a short account of the chief points to be discussed here.

The general features of the sympathetic secretion will be seen from the following experiments.

Method. The preliminary arrangements were the same as those already given for the sub-maxillary gland (p. 292) except that Jacobson's nerve was laid bare by breaking through the tympanic bulla. Light was reflected into the cavity by means of a mirror, and platinum electrodes placed on the bone close to or just touching the nerve. As will be noticed, stimulation in these circumstances produced a very unequal flow at different times. The smaller tubing spoken of (p. 292) was used except in Exps. V. and XII., in which cases a medium sized tube (62½ mm. corresponding to ·25 c.c.) was used. In the following experiments J indicates that Jacobson's nerve was stimulated.

[1] Heidenhain, *Arch. f. d. ges. Physiol.* Bd. XVII. p. 28, 1878.
[2] Eckhard, *Henle und Pfeuffer's Zeitschrift*, III. Reihe, Bd. XXVIII. 1866.
[3] Navrocki, *Studien des physiol. Instituts zu Breslau*, Hft. IV. 1868, p. 137.
[4] This *Journal*, Vol. II. p. 267, 1879.
[5] This *Journal*, Vol. VIII. p. 86, 1887.

EXPERIMENT VIII.

Sympathetic secretion from parotid gland. Effect of 18 *mgrms. atropin.*

Jan. 9, 1888. Small dog.

Stimulate sympathetic—no secretion from parotid.

1.52. Rise of saliva taken each 30 secs.

0 . 0 . 0 . 43 . 0 . 10 . 0 . 0 . 0 . 0 . 0 . 9 . 0 . 0 . 2 . 0 . 0 . 0 . 1 . 0 . 0

Sy. (c = 5·5) J. J. (for 10″) Sy*. Sy. Sy.

0 . 0 . 0 . 0 . 0 . 0

Sy. Sy.

* Greater part of the secretion in 15 seconds.

2.25. 14 . 8 . 0 . 0 . 2 . 0 . 0 . 0 . 3 . 0 . 0 . 1 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 24

J. Sy. (c = 7*) Sy. (c = 5) Sy. Sy. (c = 4) J.

1 . 8 . 1

Sy.

* Shocks weak, barely felt on tongue.

2.47. 6 . 0 . 0 . 0 . 6 . 0 . 0 . 1 . 0 . 0 . 0 . 0

J. Sy. Sy. Sy.

2.56. Stimulate Jacobson's nerve—rise of 19 mm. Inject into jugular 18 mgrms. atropin sulphate. After this neither stimulation of Jacobson's nerve nor stimulation of the sympathetic after Jacobson's nerve causes any secretion.

EXPERIMENT IX.

Sympathetic secretion from parotid.

Jan. 11, 1888. Small dog. Stimulate sympathetic, no secretion.

1.42. Stimulate Jacobson's nerve till rise 150 mm. saliva.

Rise of saliva taken each 30″.

1.50. 0 . 0 . 1½ . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0 . 0

Sy. (c = 6) Sy. Sy. Sy. (c = 4)

2.27. 0 . 54 . 40 . 3 . 0 . 0 . 12 . 2 . 0 . 0 . 0 . 3 . 0 . 0 . 0 . 0 . 0 . 28 . 3 . 2

J. J. Sy. (c = 6) Sy. (c = 5) Sy. J. Sy.

0 . 0 . 0

Sy.

2.40. 0 . 22 . 65 . 85 . 65 . 53 . 16 . 3 . 6 . 0 . 0
(22 . 65 . 85 . 65 . 53: J.; 6: Sy.)

2.47. 0 . 64 . 81 . 62 . 43 . 25 . 8 . 4 . 2 . 1 . 2 . 2 . 1 . 1 . 1 . 1 . 1 . ½ . ½
(64 . 81 . 62 . 43 . 25: J.)

5′ 4′

½ . ½ . 0 . 7 . 1 . 0 . 0 . 0 . 1 . ½ . 0 . 0 . 0 . 0 . 0
(0 . 7: Sy.; 1: Sy.; 0: Sy.; 0: Sy.)

EXPERIMENT X.

Sympathetic secretion from parotid. Effect of atropin.

Jan. 27, 1888. Medium sized dog. With scalpel cut Jacobson's nerve. The same coil was used for stimulating the two nerves, $c = 6$ gives shocks fairly felt on the tip of the tongue.

Rise of saliva taken each 30″.

15′

1.20. 0 . 0 . 0 . 0 . 0 . 35 . 14 . 1 . 3 . 0 . 0 . 0
(0: Sy. $c=6$; 35: J.; 14: Sy.; 3: Sy.; 0: Sy.; 0: Sy.)

2.2. 31 . 76—pause 11 minutes—0 . 0 . 0 . 0 . 0 . 0
(31 . 76: J.; 0 . 0: Sy.; 0 . 0: Sy.)

2.20. 16 . 39 . 3 . 1 . 3 . 2 . 1 . 0 . 0 . 0 . 13 . 24 . 0 . 27 . 10 . 4 . 1 . 4 . 0
(16 . 39: J.; 3: Sy.; 2 . 1: Sy. $c=5$; 13 . 24: J. $c=6$; 27: Sy.; 10: Sy. for 5″; 4 . 1 . 4: J.)

25 . 5 . 9 . 7 . 1 . 26 . 3
(25 . 5: Sy.; 9 . 7: J.; 26: Sy. $c=5$)

2.46. 59 . 10—pause 5 minutes—1 . 1 . 4 . 32 . 0 . 10 . 0
(59 . 10: J.; 1 . 1: Sy. $c=5$; 4 . 32: J.; 10: Sy.)

3.8. 105 . 8 . 0* . 0 . 5
(105 . 8: J.; 5: Sy.)

* Inject 5 mgrms. atropin sulphate.

No further secretion obtained by stimulating the sympathetic nor by stimulating it after stimulating Jacobson's nerve. Inject 12·5 and then 10 mgrms. of pilocarpin, no secretion nor on stimulating the sympathetic. Stimulation of the chorda tympani increases very slightly the subsequent submaxillary sympathetic saliva.

In these three experiments, the sympathetic was stimulated 17 times within a few minutes of obtaining a flow of saliva by stimulating Jacobson's nerve, and in no case did it fail to produce

a secretion. And in half-a-dozen other dogs in which I have tried the experiment I have not once observed the sympathetic to fail to cause a secretion in these circumstances. I take this to be sufficient proof that the sympathetic contains secretory fibres for the parotid gland in the dog, as it is known to do in other animals which have been experimented on.

The sympathetic secretion thus obtained from the parotid resembles closely the 'augmented' sympathetic secretion of the sub-maxillary gland, which is obtained after stimulation of the chorda tympani, differing from it chiefly in disappearing after a time entirely instead of simply declining to a minimum. The resemblance will be made sufficiently obvious—in view of the account already given of the sympathetic sub-maxillary secretion—by stating the facts with regard to the parotid secretion.

The sympathetic produces its maximum effect the sooner it is stimulated after the end of the secretion furnished by Jacobson's nerve, supposing of course that this secretion runs its normal course and ceases soon after the cessation of the stimulus to the cerebral nerve.

The weakest stimulation of Jacobson's nerve capable of producing a secretion enables the sympathetic to cause a flow of saliva; whether a stimulation of Jacobson's nerve, just below the minimal strength required to cause a secretion, can produce the same result I have not yet tried. Prolonged stimulation of the cerebral nerve has little if any greater effect on the subsequent sympathetic secretion than short stimulation, provided the latter is strong enough to cause a rapid, though brief, flow of saliva.

On successive stimulation of the sympathetic, the amount of saliva obtained rapidly falls, and usually after three stimulations of 30 seconds each, with moderately strong shocks, no further secretion results from sympathetic stimulation. A single stimulation may be sufficient for this.

If, after stimulation of Jacobson's nerve, an interval of about 10 minutes is left before the sympathetic is stimulated, it produces no secretion. The time necessary for the dying out of the effect of stimulating Jacobson's nerve no doubt varies considerably in different cases.

The secretion reaches its maximum rate of flow almost at once, in 10 to 15 seconds the rate is usually distinctly slower and then continues to fall more or less rapidly. The latent period increases with

successive stimulations, that is, the latent period is as a rule greater the slower the secretion.

When atropin is given in a dose sufficient to paralyse Jacobson's nerve, stimulation of the nerve no longer has the effect of enabling the sympathetic to produce a secretion. In the one or two experiments I have tried on the point I have failed to obtain a stage in which stimulation of the cerebral nerve, when just on the point of paralysis, and so producing little or no secretion, still enabled the sympathetic to produce one. This stage it will be remembered formed a striking feature in the atropin effect on the chorda tympani (cp. p. 311).

In Exp. X. there is some indication that atropin in paralysing Jacobson's nerve does not do away with its after-effect, inasmuch as the sympathetic gives a secretion at least three-quarters of a minute after injecting 5 mgrms. atropin, but it is possible that in so short a time Jacobson's nerve was not paralysed. In Exp. XII. (*a*) (p. 326) matters are clearer. The secretion which is going on slowly from the parotid gland, and at a fair rate from the sub-maxillary gland, is stopped in about half-a-minute by the injection of 5 mgrms. of atropin, yet 3½ minutes after the secretion has ceased stimulation of the sympathetic produces a secretion from the parotid gland, and a super-normal secretion from the sub-maxillary. Hence probably when the excitability of the gland is —in consequence of stimulation of the cerebral nerve—increased for stimuli reaching it by the sympathetic nerve, atropin whilst paralysing the cerebral secretory fibres does not do away with the state of increased excitability.

In Exp. X. (p. 322) the injection of pilocarpin after atropin, although doubtless causing some increase of blood flow through the gland, did not enable the sympathetic to produce a flow of saliva. Although it was a foregone conclusion, I tested in this and in other experiments the vasodilator effects of Jacobson's nerve after atropin had paralysed its secretory fibres; there was a flushing of the gland, although less marked than I had expected. Hence increased blood flow through the gland does not, or at any rate does not necessarily, change the ordinarily ineffective sympathetic stimulation into an effective one.

Since pilocarpin produces a secretion like that produced by stimulating the cerebral nerve, it seemed to me probable that stimulation of the sympathetic would increase the secretion brought about by injecting pilocarpin. And this proved to be the case.

EXPERIMENT XI.

Effect of stimulating the sympathetic on the pilocarpin secretion from the parotid.

Feb. 4, 1888. Large dog. Parotid duct connected with small graduated tubing. 10 mgrms. pilocarpin nitrate injected into a vein some time previously. Sympathetic stimulated with sec. coil at 10, this gives shocks moderate to tongue.

2.33. Rise of saliva taken every 30 secs. except when mentioned.

15″ each

13 . 14 . 12 . 12 . 13 . 14 . 12 . 11 . 11 . 12 . 35 . 7 . 3 . 0 . 0

Sy.

20 . 15 . 15 . 10

15″ 15″ 15″ 15″ 15″ 15″

2.42. 20 . 17 . 18 . 33 . 7 . 2 . 5 . 20 . 3 . 0 . 2 . 0 . 0 . 7 . 7 . 9

Sy. Sy. Sy.

15″ 15″

27 . 8 . 2 . 0 . 0 . 0 . 0 . 0 . 0 . 6 . 6 . 5 . 8 . 10 . 9 . empty . 7

Sy.

15″ 15″

8 . 7 . 7 . 31 . 5 . 2 . 1 . 6 . 16 . 1 . 0 . 0 . 0 . 10 . 10 . 8 . 9 . 7

Sy. Sy.

Here stimulation of the sympathetic, whilst a rather slow pilocarpin secretion is going on, increases for a short time the rate of flow very considerably; in the first stimulation it increases the rate about six times in the first 15 secs.; in the second 15 secs. the flow falls to nearly its previous rate; in the third 15 secs. the rate is about half the normal; and in the fourth 15 secs. there is no secretion at all. For half-a-minute or so there is still no secretion, and then it begins and rapidly regains or it may be exceeds its normal rate. If the sympathetic be stimulated for a shorter period so that the secretion is not stopped during the stimulation, a second short stimulation after a brief interval causes an increase in flow less than the first, and a subsequent greater fall.

Whenever the pilocarpin is causing a secretion—not too large a dose of pilocarpin having been given—an increase in the secretion can be obtained by stimulating the sympathetic. I may add that with the sub-maxillary gland of the dog the same holds, and that in this case the increase obtained is greater than that normally obtained by stimulating the sympathetic (cp. Exp. XII. (*a*) below).

Since stimulation of the sympathetic so rapidly stopped the pilocarpin secretion from the parotid, it seemed desirable to ascertain for how long it could cause this result. From observations I had previously made on the effect of stimulating the sympathetic (and the chorda) upon the pilocarpin secretion from the sub-maxillary gland of the dog[1] and the cat[2], it appeared to me that the restraining effect upon secretion was in these cases much less than in the case of the parotid of the dog. Hence I made the following experiment in which the flow of saliva from both glands was noted.

EXPERIMENT XII. (*a*).

Effect of sympathetic stimulation on the pilocarpin secretion from parotid and sub-maxillary glands.

Aug. 21, 1888. Dog. 12½ kilos. Inject pilocarpin nitrate. Rise of saliva in the tubes connected with the ducts taken every 30 secs. Sec. coil at 8 gives shocks fairly strong to tongue.

2.25.	Sub-maxillary	24	25	24	25	23	23	–	25	54	9	3	2	1	0			
	Parotid	5	5	4	5	5	4	5	5	9	2	0	0	0	0			
										Sy. $c=8$								
	Sub-maxillary	0	0	1	3	11	19	29	32	32	–	30	32	29	29			
	Parotid	0	0	0	0	0	0	1	2	2	2	3	4	4	5			
		Sy. $c=8$																
	Sub-maxillary	27	51	4	1	0	0	1	1	1	1	2	1	1	1	0	0	1	0
	Parotid	6	14	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
										Sy. $c=7$									
	Sub-maxillary	1	0	0	–	5	7	10	12	16	18	21	22	21	23			
	Parotid	0	0	–2	–1	0	0	0	0	0	0	1	1	1	1			
		Sy. $c=7$																
	Sub-maxillary	23	21	20	18	–	25	25	24	21	23	19	20					
	Parotid	1	2	1	1	1	1	1	1	2	1	2	2					
	Sub-maxillary	15	3	0	0	0	–1	–2	–1	–1	25	1	1	0				
	Parotid	1	1	0	0	0	0	0	0	0	5	0	0	0				
		Inject 5 mgrms. atropin									Sy.							

[1] *Journal of Anat. and Physiol.* Vol. XI. p. 177, 1876.
[2] This *Journal*, Vol. I. p. 342, 1878.

After this stimulation of the sympathetic gives no secretion from the parotid and a scanty one only from the sub-maxillary gland.

For rest of experiment see p. 307.

It will be seen that on stimulation of the sympathetic for 10 minutes, the secretion from the parotid stopped in a minute and a half, or less, and there was no further secretion during the remainder of the stimulation, i.e. for 8½ minutes. Further there was no secretion for three minutes after the end of the stimulation, and lastly that when the secretion did begin it was distinctly less in amount than previous to the stimulation.

It will also be seen that in the sub-maxillary secretion, prolonged sympathetic stimulation produces much less effect. In the first stimulation for four minutes, it is true that there is no saliva for the last 1½ minutes, but the secretion begins within half a minute of the cessation of the stimulus and soon exceeds the rate obtaining previous to the stimulation. In the second stimulation of 10 minutes, there was now and then no appreciable secretion, but it can hardly be said to have definitely stopped; within a minute of the cessation of the stimulus the flow of saliva begins, and compared to the previous occasion slowly increases in rate up to about that obtaining at the beginning of the experiment, that is to say—allowing for a gradual decrease of secretion during the experiment—to a somewhat greater rate than it would have had but for the stimulation.

The very close resemblance between the effects of stimulating the chorda tympani, upon the sympathetic secretion of the sub-maxillary gland, and the effects of stimulating Jacobson's nerve, upon the sympathetic secretion of the parotid gland, renders it, I think, certain that the same cause is at work in the two cases; and we may conclude in general terms that the sympathetic secretion obtained from the parotid after previous stimulation of Jacobson's nerve is due to an increased irritability of the gland produced by the secretory fibres of Jacobson's nerve. I am inclined to think that in all cases, the sympathetic causes the parotid to secrete, but that this secretion is normally so thick as to clog up the ductules and ducts. At any rate, stimulation of the sympathetic which has caused no flow of saliva, has in all cases which I have examined been accompanied by a considerable distension of many of the ductules and lobular ducts with secretion. If this is so, the flow of saliva which is obtained by stimulating the sympathetic, subsequent to stimulation of Jacobson's nerve, would be due to the saliva being more dilute and

so offering less resistance to being driven out of the gland. I am making further observations on this point.

CONCLUDING REMARKS.

We have seen that stimulation of the cerebral nerve of either the sub-maxillary, the sub-lingual, or of the parotid gland, increases the irritability of the gland for impulses reaching it by the sympathetic nerve. In this statement the phrase 'increase of irritability' is used in the most general manner, and means little more than that in this state the sympathetic with a given stimulus produces a greater secretion than normal. The phrase in fact simply states the result and does not explain it.

It does not seem to me to be at present very profitable to discuss the matter at length, for to do so involves the question of whether there is or is not more than one kind of nerve fibre in secretory nerves. And there is to my mind no conclusive evidence either one way or the other. But although a discussion of the question is as matters stand premature, it is well to bear in mind the possible explanations. Besides those which involve the presence of different kinds of nerve fibres, a possible explanation of the facts here given is that the chorda tympani and the sympathetic nerve fibres supply different gland cells, that when either nerve is stimulated there is an irradiation of impulses of less intensity to cells in the neighbourhood of those directly affected, that on stimulation of the chorda tympani the cells connected with it are left for a time in a state of weak excitation, so that the irradiation of impulses reaching the gland by the sympathetic is much greater than normal and these irradiating impulses being weak lead to a more fluid secretion.

This, it is true, involves the difficulty of the way in which an irradiation of impulses can take place; an irradiation either by nervous structures or by gland cells—and especially perhaps the former—is not very easy as the facts stand to clearly comprehend.

Feb. 14, 1889.

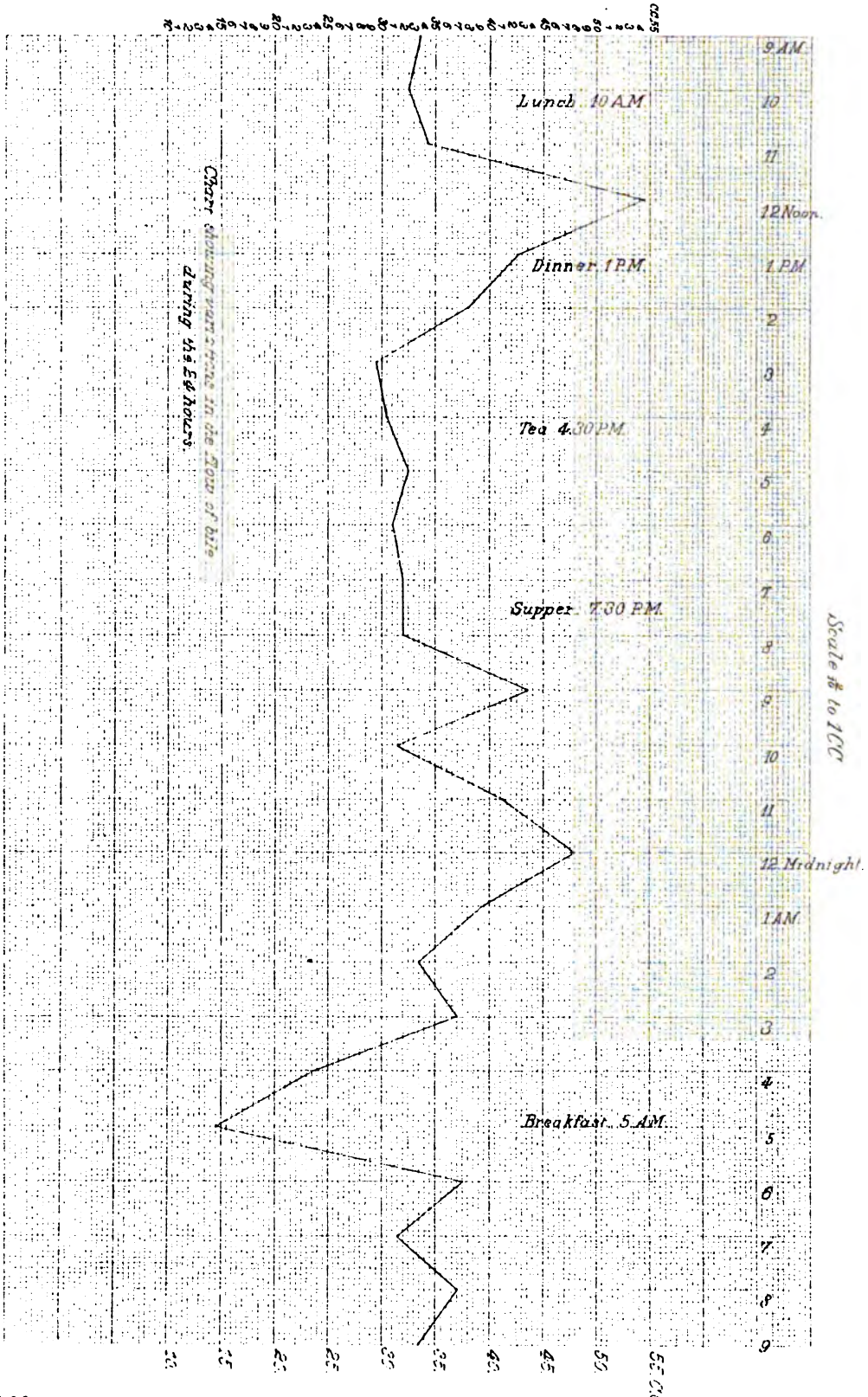


Chart showing variations in the flow of bile during 24 hours.

S.M.C. del.

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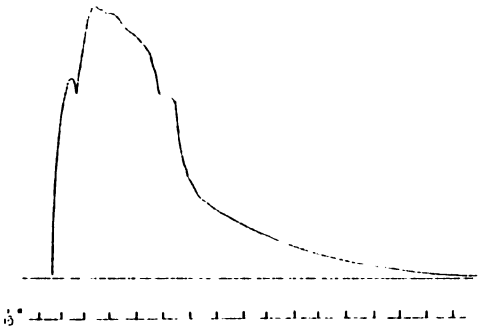

Fig. 1.

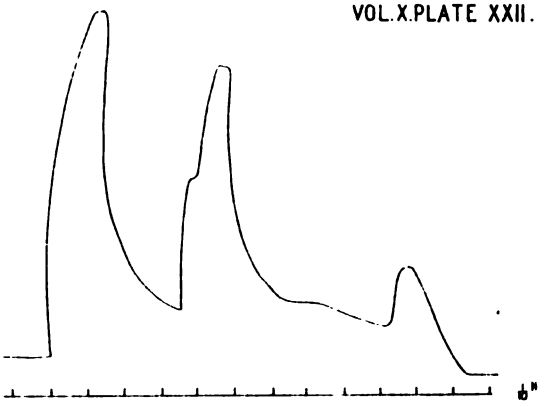

Fig. 3

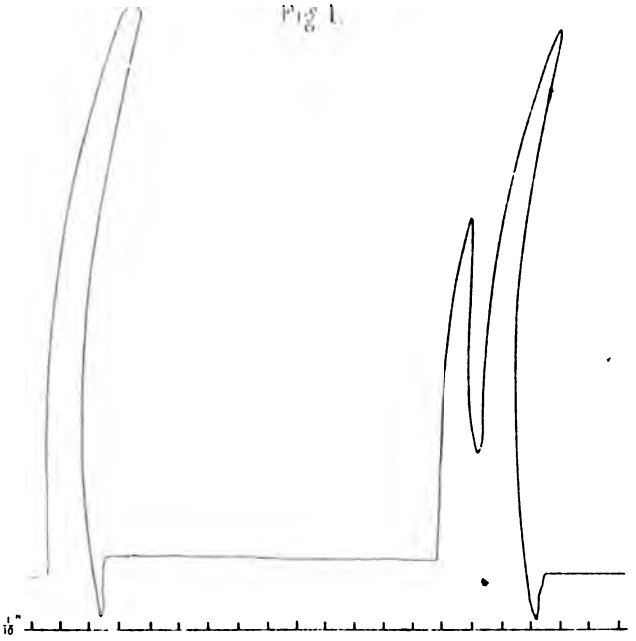

Fig. 4.

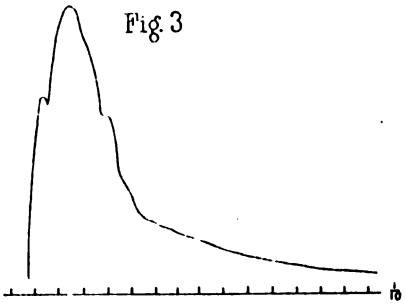

Fig. 2.

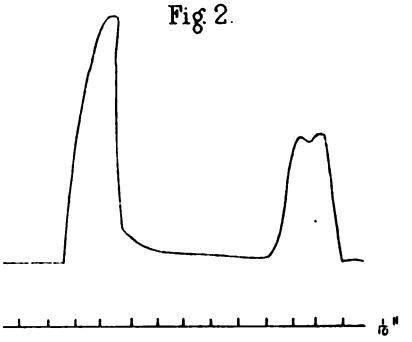

Fig. 5

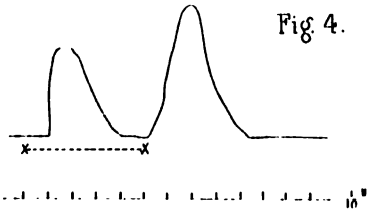

Fig. 6.

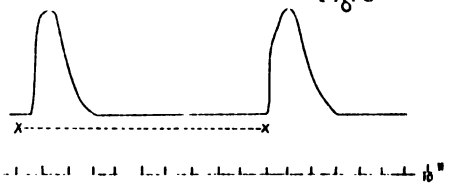

Fig. 7.

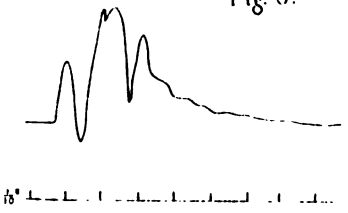

Fig. 8.

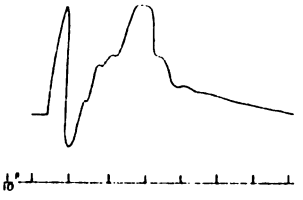

Fig. 9.

Fig. 10

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THE COAGULATION QUESTION. By L. C. WOOLDRIDGE, M.D., D.Sc., *Joint Lecturer on Physiology and Assistant Physician to Guy's Hospital.*

The terribly sad event, the untimely death of the author on June 6, by which Physiology has been robbed of a bright and enthusiastic inquirer, has laid on me the duty of carrying out by myself the final revise of this paper. It had already been revised by the author; and I have endeavoured to make as few changes as possible.

M. FOSTER.

In a paper read before the Royal Society in April 1888, and recently in this Journal, Dr Halliburton has published his belief that the fibrin ferment is a cell globulin, and also a somewhat extensive criticism of my work on coagulation.

In the following discussion of the chief points which Dr Halliburton has raised against my work and views I will first take the last point handled by Dr Halliburton in his "criticism."

An important feature in my work on coagulation is the production of intravascular clotting by the substances I call "tissue fibrinogens."

As an explanation of this intravascular clotting, Dr Halliburton said, in his Royal Society paper, there was fair reason to suppose the solutions of these substances were of a very slimy nature, so that they would, when injected, naturally block up the small vessels and thus cause coagulation; just as an iron wire will cause coagulation when introduced into the sac of an aneurysm.

From the very first papers I had published on this subject (1886) it was clearly impossible that this suggestion could be correct, and in the light of what I had subsequently published, previous to Dr Halliburton's first paper, it was manifestly out of the question. With the greatest ease Dr Halliburton could, by repeating my experiments, have seen whether his assertions were true or not. This he has now done, and he withdraws his observations unreservedly.

In his paper before the Royal Society, Dr Halliburton stated also that I had spoken of a substance, B-fibrinogen, in my abstract of the Croonian lecture, and that this substance had disappeared from the paper which was published in Ludwig's *Festschrift* or had become identical with Hammarsten's fibrinogen. Now since a large part of my paper in Ludwig's *Festschrift* was devoted to describing B-fibrinogen, and the differences between it and Hammarsten's fibrinogen, Dr Halliburton's assertions were quite unintelligible to me.

In my researches on coagulation I lay great stress on a substance which separates from peptone-blood-plasma on cooling; I call it A-fibrinogen. I have taken the greatest and most conscientious trouble to investigate this substance, and I regard it, and I am sure with truth, as of the greatest importance in coagulation processes. Now Dr Halliburton plainly indicated in his first paper that this precipitate was merely an albumose mixed with the peptone injected and was in no way a constituent of the blood. The principal point in his criticism was, and is still, that solutions of Witte's and Grubler's peptone yield on cooling a precipitate of rounded granules. I pointed out that the peptone I use (Grubler's) gives no precipitate on cooling, and it disappears entirely from the blood. I was, of course, aware at the time when I first found the "cold body" (A-fibrinogen) that peptone (using the word as a generic term) had been obtained in rounded granules (Schmidt-Mulheim), and also that other proteids, i.e. vegetable albumen and paraglobulin, could be obtained in rounded granules; for I had worked for a long time at Leipzig at crystallized proteids, the first step towards crystallization always being the production of rounded granules. The mere fact that A-fibrinogen can be obtained in rounded granules is of course no more entirely characteristic of it than would the statement that a body was crystallizable suffice to distinguish it. But from the very first there could be no doubt that this substance was not peptone. I had seen it for a long time before recognizing what it meant. As I saw it, its appearance was precisely similar to a fibrinous clot, and for a long time I thought it was a partial coagulation of the plasma due to the

presence of leucocytes. I was struck by the fact however that this clot only appeared when I kept my plasma overnight in ice and that it was absent from my plasma when kept in the warm room. Its characters, both chemical and physical, and its influence on coagulation, I have described in detail, in my papers "Ueber einen neuen Stoff, etc."[1]; "On a new constituent of the plasma"[2]; in Ludwig's *Festschrift*, and "Beiträge zur Frage der Gerinnung"[3]. If Dr Halliburton had suggested that A-fibrinogen was merely fibrin it would have been intelligible but not correct. But although it is difficult from Dr Halliburton's statements[4] that cooling peptonised serum and other solutions of peptone give a precipitate exactly similar to that of A-fibrinogen (Wooldridge), and that, as Neumeister has shown, the albumose or peptone injected "exists as such or in a loosely combined condition in the blood for a time," to conceive that he meant or means anything else than that my A-fibrinogen is but such precipitated albumose, we are now to understand (p. 280, the same on which the above statements are made) he means nothing of the sort. All he means is that albumose alters the normal proteids of the plasma. This is quite a different view, and is of course a necessary conclusion from my own experiments and publications. In fact Dr Halliburton tries to discredit my observations on this subject by

I. Hinting that it is nothing but albumose precipitated by cold, indeed stating, page 278, § 2, "that the occurrence of the so-called A-fibrinogen in peptone plasma is produced by the peptone, is supported by the fact that if one takes a solution of Witte's peptone and cools it to 0° C. a precipitate is produced consisting of rounded granules having very much the appearance of blood tablets."

II. Asserting that nevertheless it is not albumose, is not the actual precipitate from the peptone injected.

The flagrant contradiction of these two ways of looking at the subject is only consummated by Dr Halliburton's suddenly dropping the subject he raises, viz. what is the nature of the body I call A-fibrinogen? But he proceeds to add remarks the justification for which rests on his ignorance of my researches on this subject.

[1] du Bois' *Archiv*, 1884.
[2] *Proc. Roy. Soc.* 1885.
[3] du Bois' *Archiv*, 1888.
[4] *Proc. Roy. Soc.* Vol. XLIV. p. 266, and this *Journal*, p. 280.

For instance Dr Halliburton complains that in my short answer to him in the *Proceedings of the Royal Society*, I do not give a list of the properties of A-fibrinogen. Am I compelled to rewrite all my descriptions and facts because Dr Halliburton will not take the trouble to read them? On page 278 of the Journal paper Dr Halliburton says the following in reference to the occurrence of A-fibrinogen in salted plasma:—"The statement that sodium chloride plasma contains A-fibrinogen is thus a matter of pure inference: and the reasoning adopted by Wooldridge is a very obvious case of reasoning in a circle as follows:—this form of salted plasma coagulates spontaneously, therefore it must contain fibrinogen A, and because this plasma contains fibrinogen A therefore it coagulates spontaneously."

What I have really said on this subject is as follows: Peptone plasma free from ferment and corpuscles:—

1. Yields on cooling a substance which can be collected and examined, A-fibrinogen.

2. Clots spontaneously, i.e. by means not[1] fibrin factors.

3. When it clots spontaneously yields fibrin ferment.

4. Cooled, and with A-fibrinogen thus removed, loses the power of spontaneous coagulation and of forming fibrin ferment.

5. Treated with sodium chloride solution so that the mixture contains 4 to 5°/₀ of the salt, yields no precipitate on cooling and retains the power of spontaneous coagulation.

6. If treated with $MgSo_4$, as I have described, a precipitate is formed in the plasma closely resembling the cold precipitate, A-fibrinogen.

7. After the removal of this precipitate the plasma no longer coagulates spontaneously and forms no fibrin ferment.

To judge of the action of salts on plasma we must first have it. Normal blood of the dog (i.e. blood the moment it leaves the vessels) treated severally with 10°/₀ NaCl solution and $MgSo_4$ solution yields plasmata exactly corresponding as regards spontaneous coagulability and *formation* of fibrin ferment to those obtained by the action of salts on peptone plasma.

Consequently I consider I am justified in the deduction that A-fibrinogen is present in salted plasma.

In his own paper Dr Halliburton says, "Magnesium sulphate plasma is generally the slowest to coagulate when simply diluted with

[1] ? independently of. *Ed.*

water: this is *no doubt* because some of the fibrinogen of the plasma is precipitated by the use of this salt." It is not clear why Dr Halliburton says "no doubt." It is precisely the statement I have made in consequence of my study of the action of magnesium sulphate on peptone plasma. Nor is it clear why, with his views, Dr Halliburton thinks the removal of this fibrinogen should delay the occurrence of clotting. Has he any evidence to show that a slight diminution in the quantity of fibrinogen makes the rest clot slower with ferment? Why should it?

Now there is this difference between the two kinds of salted plasma in the dog. The sodium chloride plasma clots in from 3 to 10 minutes on simple dilution. The magnesium sulphate plasma does not clot for 48 hours. Such a striking difference is only explicable by the assumption that the fibrinogen removed by the $MgSo_4$ has a special power of initiating coagulation.

What I have said is that NaCl plasma and uncooled peptone plasma contain a special fibrinogen (A-fibrinogen) which has the power of initiating coagulation.

Dr Halliburton first tries to throw ridicule on my results, and then for his own purposes practically admits they are true.

He states in his paper in the Journal that *I infer that solutions of tissue fibrinogen cause clotting in extravascular plasma*, from its action on intravascular blood. But this again is quite inaccurate.

In my paper in Ludwig's *Festschrift*, and in a paper published in March, 1888, in du Bois' *Archiv*, I state and describe fully the action of tissue fibrinogen on extravascular plasma.

I quote from Ludwig's *Festschrift* (which Dr Halliburton frequently refers to) "Diese Stoffe (Gewebes-fibrinogen) bewirken nicht nur Gerinnung im extravascularen Plasma wie Pepton-plasma sondern sie bringen auch beim Einspritzen u. s. w. Die genannten Stoffe sind ähnlich wie Lymphkörperchen und *Lecithin* gänzlich ohne Wirkung auf stark verdünntes Bittersalzplasma—bringen sie dagegen extravasculares Plasma zur Gerinnung so entsteht gleichzeitig viel Ferment."

I think the illustrations I have given above indicate fairly the nature of Dr Halliburton's criticism.

Having sufficiently dealt with what I must characterize as a travesty of my researches, I now pass to a consideration of the actual observations of Dr Halliburton. Dr Halliburton, to his own satisfaction, conclusively overturns all my work by the following highly remarkable

dictum. "Now Dr Wooldridge admits too much, he admits that the fibrin ferment causes coagulation" and, "The formation of fibrin either is or is not a ferment action, it cannot be both."

Sugar is formed from starch by ferment action; does Dr Halliburton think it can be formed in no other way? In such a state of mind does Dr Halliburton approach the coagulation question. Truly I admit that fibrin ferment sometimes causes the coagulation of fibrinogen; to state that fibrin cannot be formed in any other way is to beg the question at issue, and consequently to assume most unwarrantably perfect knowledge of the whole process of coagulation.

The destruction my observations undergo, in Dr Halliburton's hands, when he treats them experimentally, is quite on a par with that treated logically.

He completely "upsets one of the very foundations on which" my theory is constructed.

He describes six experiments in which he prepares according to Schmidt's method solutions of fibrin ferment from lymphatic glands. He consequently "feels justified in concluding that the cells of lymph glands yield as one of their disintegration products a substance which has the same properties as fibrin ferment." "This is a fact which has been observed by others; it is however denied by Wooldridge." I have of course never denied in the least that leucocytes might as the result of disintegration yield fibrin ferment; quite the contrary. The real statement I have made is reproduced in the very same sentence by Dr Halliburton himself, and among the "others" which he quotes as supporting his view and diametrically opposed to me, is Rauschenbach.

Dr Halliburton's own work on leucocytes is in many respects an incomplete repetition of Rauschenbach's.

Rauschenbach, a pupil of Alexander Schmidt, repeated and extended my original observations, using cooled plasma. He is the originator of the statement that the leucocytes from lymph glands contain no fibrin ferment and that they have no action on certain fibrinogen solutions. He is the originator of the statement that there are "mother substances" of the fibrin ferment. From my own papers eight years ago and from Rauschenbach's onwards there has been a complete revolution in the coagulation question.

I quote from Rauschenbach[1].

[1] *Protoplasma und Blutplasma*, Dorpat, 1882.

"When the cells are treated with distilled water and immediately filtered, ten parts of the filtrate with one part of salt plasma (the test fluid) gave signs of a weak fermentative activity (clotting in 4 or 5 hours)" (p. 27).

"The slimy mass of leucocytes which had been produced by the action of salts, treated with alcohol yielded only traces of fibrin ferment" (p. 25).

"After the cells had been acted on by distilled water for many hours an active extract was obtained; heating to 50° quickened the formation of this active extract" (p. 27).

"Whether the traces of fibrin ferment which even the freshest collection of cells contains, and which pass into the watery extract of the alcohol coagulum and into the rapidly filtered watery extract of the cells themselves, are of vital origin or are formed during the pressing out and filtration of the mass, I must leave for the present undecided" (p. 28).

"I must expressly emphasize here that fresh pressed out cell fluid, in a quite undiluted condition exerted no perceptible action whatever on my fibrinogen fluids nearly free from paraglobulin. The same is true of the watery extract obtained immediately after pressing out the cells" (p. 30).

My observations in regard to the absence of ferment from fresh isolated leucocytes are quite in accord with those of Rauschenbach. As Dr Halliburton quotes him one would gather that this observer's statements were quite in harmony with those of Dr Halliburton and opposed to mine. Whereas the exact contrary is the case.

Now these observations of Rauschenbach clearly point to the fact that fresh isolated leucocytes contain none or dubious traces of fibrin ferment; that by artificially breaking them up fibrin ferment may be formed. Dr Halliburton, in dealing with me, says that all my complicated and in his eyes apparently ridiculous observations fall to the ground because the leucocytes contain such a powerful fibrin ferment. He says, speaking of peptone plasma: "Leucocytes contain a very powerful ferment, therefore they cause clotting in peptone plasma, whereas serum and Schmidt's ferment are not so powerful."

There is not a particle of evidence in the whole of Dr Halliburton's paper in support of this statement—the one experimental point he endeavours to make against me.

From the six experiments I have alluded to it is impossible to judge whether there is any fibrin ferment present at all. Since the only approxi-

mately reliable experiment, with strong magnesium sulphate plasma, is spoilt and rendered dubious by the very slight dilution of the plasma; and as no weight or measures or comparisons with serum are given, there is not even an attempt to prove his statement.

Lymph cells and leucocytes yield very active "preparations of fibrin ferment," says Dr Halliburton; although he in no way proves it, it may be true. But if it should prove to be true that leucocytes, without any other organic addition, can be made to yield active preparations of fibrin ferment, this would not in the least alter or injure what I have said on coagulation.

I have said they do not contain it, I have not said they might not, by various destructive processes, be made to yield it. On the contrary I have always said that when they cause clotting in peptone plasma, they also cause the appearance of fibrin ferment. Whether any other form of destroying the cells may cause the formation of fibrin ferment I cannot personally say.

Dr Halliburton seems to think that a leucocyte in a lymphatic gland alive and well is the same thing as a leucocyte treated with various strong salt solutions, dialysed, treated with alcohol, distilled water and so on. It is of course easy to say, as Dr Halliburton does, that leucocytes cause the clotting of the plasma because they contain a very active ferment. His experiments prove nothing of the sort. My own observations are quite opposed to this statement. We can only judge of the activity of a ferment solution by the rapidity with which it causes the clotting of a test fluid.

Now solutions of fibrin ferment which cause clotting in strongly diluted strong magnesium sulphate plasma in two to three minutes cause when added to peptone plasma in the same proportions no clotting whatever or only traces after hours. Yet leucocytes added to the same peptone plasma cause clotting in a few minutes. If these experiments mean anything, they mean that the leucocytes act quite differently to strong fibrin ferment. And it was perfectly apparent from my first experiments published in 1881 that the interaction which occurs between the leucocytes and plasma was of a totally different nature to a fermentative process.

Now concerning Dr Halliburton's observations I would say the following.

They leave the chemical aspect of coagulation quite untouched; his theory is therefore naturally simpler than mine. For to say that coagulation is due to a ferment is merely to envelope the whole

question in the hopeless mist in which it has been for the last twenty years.

Dr Halliburton says the white corpuscles liberate fibrin ferment when blood is shed, and he says his theory explains why the blood coagulates outside the vessels. This is, of course, the old theory of Alexander Schmidt; a theory which has long been shown by its author and his pupils to be hopelessly untenable. It was shown to be impossible in my first papers in 1881.

Dr Halliburton's repetitions of Schmidt's experiments and his deductions therefrom are as follows. By destroying lymph cells in various ways he obtains solutions which he says contain the fibrin ferment. Therefore he says when the white corpuscles leave the blood vessels they break up and yield this ferment. Finally, he says, leucocytes contain a very active ferment, a much more active ferment than serum.

Now if, for the sake of argument, we accept these observations as correct, it is clear that there is a very large gap between Dr Halliburton's deductions and his observations, because we know with certainty that white blood corpuscles when they leave the vessels contain no fibrin ferment. Leucocytes and white blood corpuscles are then according to this totally different, if Dr Halliburton's statements are correct and he means what he says. Under these circumstances, therefore, the ferment theory as propounded and maintained by Schmidt falls to the ground in Dr Halliburton's hands. He himself does not appear to grasp the fact that Schmidt was quite aware that the leucocytes in the blood contained no fibrin ferment and that he accordingly put forward the hypothesis that a specific form of protoplasmic change occurred, which led to the development of ferment from the disintegrated corpuscles.

Now, unfortunately, the views of Schmidt and Hammarsten edited by Dr Halliburton are supported by him by an experimental method which is open to fatal objections.

He uses, as a test for his supposed fibrin ferment solutions, a fluid which he says clots rapidly with substances which are not fibrin ferment. It is therefore extremely difficult for him to know when he is dealing with the ferment and the non-ferment. He says that because his solutions cause clotting in his test plasma it cannot be concluded that they contain ferment because other substances, e.g. muscle, cause rapid clotting. (In his first paper in the Royal Society he omits this.) It is therefore not at all clear how he knows he is dealing with fibrin

ferment at all. His identification of the ferment would appear to depend on the temperature at which his solutions lose their coagulating activity, and even this is, according to his own statement, a somewhat precarious method of identification.

The fibrin ferment is, he asserts, "cell globulin," a substance containing no phosphorus. "It is a true ferment, an unstable substance, the product of a living cell, and that induces changes in the substances with which it comes in contact by a catalytic action, i.e. without itself apparently participating in the chemical changes its presence induces." Dr Halliburton, it may be seen, is very precise on the subject. But as a matter of fact all this rests purely on his ipse dixit.

Long before he took up the subject of coagulation it had been abundantly shown that all kinds of different substances when added to plasma cause coagulation. These substances were not fibrin ferment. And therefore it became of the utmost necessity to adopt the greatest precautions in testing for this substance. The two fluids which afford reasonable certainty that we are dealing with fibrin ferment are (1) the test fluid of Alexander Schmidt, i.e. highly diluted strong magnesium sulphate plasma, and (2) the fibrinogen of Hammarsten, i.e. the fibrinogen prepared from the strong $MgSo_4$ plasma of the horse by repeated precipitation and re-solution. They afford a reasonable certainty of the existence of ferment because so far as is known they have little or no tendency to clot with "other substances." Dr Halliburton appears very seldom to use these test fluids. As I have pointed out before, the serous fluids which he employs are very unreliable, they vary in different cases, although as a general rule they resemble Hammarsten's fibrinogen in being easily affected by ferment and not by "other substances."

It must be remembered that the original and continued belief in the fibrin ferment depends almost entirely on our being able to separate it from proteids. Its recognition by Schmidt depended on this. The whole of Hammarsten's work depended on this. If it cannot be separated from proteid then the proof that it produces "changes without itself participating in the chemical changes" becomes an exceedingly difficult one and has never yet been presented to science.

Dr Halliburton says "that his ferment does not participate, but I cannot see that he has afforded any material proof whatever. Indeed he is not clear that his proteid solutions, which he calls ferments, do not influence the amount of fibrin formed, and he affords no direct proof whatever that they are not materially altered. He does not give

convincing evidence that the various substances he speaks of as "fibrin ferment" are one and the same body. It is of course extremely hazardous to assert that one proteid is identical with another. But first there is one point Dr Halliburton seems to have omitted to profit by. His cell globulin from lymph glands is "quite free from phosphorus." This is the first proteid, if this is true, ever found free from phosphorus (Bunge Ritthausen). It is a pity he has not applied this method of identification to his other cell globulins.

Then Dr Halliburton makes a very remarkable statement about the fibrin ferment which is entirely opposed to all previous experience. He says that even ½ or 1°/₀ solutions of sodium chloride hinder very considerably the action of the ferment. I hope Dr Halliburton has made direct observations on this point, since it is another of the sweeping and unsupported statements he has made concerning my work. The action of sodium chloride has been very fully worked out by the Dorpat school. The presence of salt solution up to 3°/₀ causes not a hinderance but a decided increased rapidity in fermentative coagulation. Against this well-known fact he brings no experimental evidence. The sole original point which Dr Halliburton has made is that the fibrin ferment is a proteid. All the observations he gives on clotting have been made before. We have known for years that leucocytes from lymph glands cause clotting, that extracts containing proteid from leucocytes cause clotting, that a proteid from serum causes clotting, and it is still for Dr Halliburton to prove that these substances are the fibrin ferment, or that they act as an enzyme at all, and even then we shall not have got much further in the knowledge of the intimate nature of coagulation.

How extremely little value can be placed on the effect of heating as a test for an enzyme can be seen from an earlier observation I have long ago recorded. Tissue fibrinogen causes intravascular clotting; after the solutions have been boiled they lose this power. Yet they are just as active on extravascular plasma as peptone plasma.

In conclusion then, Dr Halliburton's sole experimental point against me is that leucocytes cause clotting in peptone plasma because they contain a ferment so very much more active than the ferment of Schmidt or than serum. He does not, however, adduce one single observation which proves that leucocytes contain or even yield any ferment whatever, and there is no attempt to prove that their alleged ferment is so very active.

The observer he quotes in support of his views flatly contradicts him. Surely an observer in Dr Halliburton's position, who brings such sweeping criticism to bear on my work, is bound to show that his one observation "which completely upsets one of the very foundations upon which my theory rests" is a true and correct one. But at present Dr Halliburton has only made the assertion, he has not attempted to prove it.

FURTHER OBSERVATIONS ON THE HISTOLOGY AND FUNCTION OF THE MAMMALIAN SYMPATHETIC GANGLIA. By W. HALE WHITE, M.D.

(*From the Laboratory at Guy's Hospital.*)

In this *Journal*, Vol. VIII. No. 2, I tried to show, Firstly:—that human adult superior cervical ganglia vary as much in size as the largest and smallest of other mammals, and that the size of the ganglion in other mammals varies directly as the size of the animal. Secondly:—that human superior cervical ganglia exhibit granular pigmented atrophic cells much more frequently than those of other mammals; that this condition though present to some degree in monkeys, diminishes regularly as we descend in the mammalian scale, till at last it is not seen. Thirdly:—that human fœtal superior cervical ganglia do not show any of these changes in their nerve cells. These facts seemed to me to demonstrate that the superior cervical ganglion is becoming less and less important functionally the higher we ascend in the animal scale, till in the human adult its minimum of importance is reached. It is in fact an atrophied degenerate organ like the coccyx or appendix cæci.

Since the publication of that paper I have made some further observations on this subject.

In order to make sure that the pigmented, granular, atrophic, degenerate appearance of the cells could not be due to the method of preparation of the specimen, I examined in the fresh state the superior cervical ganglion of ten persons dying of various diseases, and found that the nerve cells showed exactly the same appearances as those which I have described as being present in the nerve cells of ganglia which have been hardened in Müller's fluid. This condition of nerve cell in the human subject has been found by many observers, such as Foa (*Rivista Clinica di Bologna*, 1874), Giovanni (*Patologia del*

Simpatico, Milano, 1876), Luminoff (*Virchow's Archiv*, Bd. 61, § 145), Da Costa and Longstreath (*American Journal of Medical Sciences*, July, 1880), Long Fox (*Influence of the Sympathetic on Disease*), and Saundby (*British Medical Journal*, Jan. 13, 1883). It is extremely unlikely that all these observers prepared their specimens in the same way, and yet they found exactly the same changes as I have done both in prepared and fresh specimens. Therefore we must I think admit that this condition of cells is present during life.

Wishing to find out whether the age of the subject bore any relationship to the condition of the superior cervical ganglion, I examined this organ taken from twenty-two different people dying of different diseases, wrote a description of each specimen without knowing the age or cause of death of the patient, and then arranged the descriptions according to the ages of the patients. The result of this is shown in table A at the end of this paper. It will be observed that four children of 10 years of age and under were examined, and in all of them the nerve cells were well formed, neither shrunken, granular nor pigmented, and in every respect looked like ordinary nerve cells. In the remaining eighteen patients, all of them adults over twenty-one, the nerve cells were not like those of children save in a single case; they were in all others either granular, shrunken, non-nucleated, or very few in number, and had not by any means the usual appearance of nerve cells. It will also be noticed that the cells are furthest removed from the normal type in the oldest people, for example, the nerve cells from the superior cervical ganglion of the three people who were seventy or over, were very scanty, and were nothing more than small granular masses of pigment. In my last paper in this *Journal* I described one superior cervical ganglion from a child, and its nerve cells were quite normal. Out of the 41 specimens there described, all but nine were taken from persons who died of diseases which very rarely occur in children. If we take out these nine we have thirty-two specimens left, one of which was a child with normal nerve cells, but in all the remaining thirty-one the cells were unlike the normal type. Thus, putting the lists from each of the papers together we have forty-nine adults examined, among whom only one showed entirely normal nerve cells in the superior cervical ganglion, and five children examined, all of whom showed entirely normal nerve cells. In my last paper I demonstrated that in each of ten human fœtuses the cells of the superior cervical ganglia looked exactly like normal nerve cells, except that they were rather small, which was probably due to their not being fully grown. Schenk and

Birdsall[1] have shown that the sympathetic ganglia are developed, both in man and other mammals, by an outgrowth from the central nervous system, and that the cells of the sympathetic ganglia are at first indistinguishable from those of the central nervous system, which however grow more rapidly than the sympathetic cells, so that soon the sympathetic cells may be distinguished by their being smaller.

I also proved that the superior cervical ganglion varied more in size in adult man than any other organ (except perhaps other ganglia) in the body. Although there are a few cases on record in which the cervical sympathetic ganglia have been implicated in a tumour, in none were there any symptoms save those which could be referred to the destruction of the nerve fibres passing through the ganglion, and no disease has ever been shown to be due to a lesion, either intrinsic or extrinsic of the cells of these ganglia.

All these facts seem to me to afford additional proof of my contention that the superior cervical ganglion is in the human fœtus an organ having some function, but that soon after birth the ganglion begins to degenerate, and this degeneration continues till, towards the end of life, the nerve cells are atrophied and functionless.

It will be noticed, that in fœtuses and children none of the cells are pigmented, but that at the age at which other signs of degeneration such as the appearance of a granular condition, shrinking, absence of nucleus &c., begin to appear, pigmentation is first seen. This may perhaps mean that we ought to regard the pigmentation as evidence of degeneration. The cause of death has no influence on the condition of the nerve cells.

In 21 out of the 22 patients whose superior cervical ganglia are described at the end of this paper, I examined the cervical sympathetic nerve between the superior and middle ganglia, and in every case it was perfectly healthy, being composed of grey fibres with a few small white ones mixed with them. It is noteworthy that if the sections showed among the nerve fibres a few nerve cells these were pigmented, granular, and degenerate. That the human sympathetic nerve in the neck is functionally active is well known from the experiments of Prof. R. Wagner upon the head of a decapitated criminal, whose pupil was made to dilate by stimulation of the cut distal end of the cervical sympathetic nerve. Also there are numerous cases on record in which the nerve has been destroyed by aneurisms, or growths, or cut by

[1] *Mittheilungen aus dem Embryologischen Institute*, Bd. 1, Wien, 1880.

injuries, and there is even one recorded by Israel (*Berl. Klin. Woch.* 1888, § 120) in which a piece of the nerve was excised during a surgical operation. All these cases show that the sympathetic nerve in the neck in man possesses exactly the same function as it does in animals. Möebius (*Berl. Klin. Woch.* 1884, § 234, u. folg.) has published an important collection of such cases.

I therefore submit the conclusion that although in adult man the superior cervical ganglion is functionless, the cervical sympathetic nerve has the same functions as in the lower animals.

In my previous paper I gave a list showing the results of the examination of the superior cervical ganglia taken from forty-six mammals lower in the scale than man.

I have in addition examined twenty-three more. The results will be seen in table B at the end of this paper. It will be noticed that in all the lower animals the nerve cells are of the normal type save in one of the Catarrhine apes. This confirms the result previously arrived at, namely, that whilst adult man presents a very large proportion of granular degenerate cells, in monkeys there are very few and in the mammals below them hardly any.

The sections of this ganglion from the lower animals also show that the size of the ganglion varies directly as the size of the animal.

All these additional facts confirm my previous contention that the superior cervical ganglion in the lower mammals has some function; in monkeys it has begun to lose this function, and in adult man it is entirely functionless.

The spotted hyæna, the spectacled bear, the crab-eating raccoon, Michies' tufted deer and the white lipped peccary were all described as aged, and the bear was very old, yet all these animals showed well-developed non-degenerate nerve cells. This is in striking contrast to the minute, non-nucleated masses of granular pigment, which we have seen represent the nerve cells in the superior cervical ganglia of elderly human beings, and is further evidence that, in adult man, these ganglia have lost whatever function they may have had in the lower animals.

In my second series of sections as in my first I have sometimes found a large number of leucocytes; I cannot say what their significance may be.

In 19 out of the 23 animals examined, sections of the cervical sympathetic nerve between middle and superior cervical ganglion were cut, and they were always found to be perfectly healthy and quite indistinguishable from the corresponding human nerve.

There will be found tabulated in Table C at the end of this paper, the result of an examination of 33 human semilunar ganglia, taken indiscriminately from persons dying of various diseases. Of the 33, three came from children, and in all of them the ganglion cells were excellent examples of normal nerve cells, some of them showing processes as distinct as those of the cells of the spinal cord. In six only of the remaining 30 were all the nerve cells normal; all the other ganglia showed more or less degeneration of their cells which in many sections were reduced to minute masses of non-nucleated, granular pigment, free in the middle of the capsule.

Often it is particularly mentioned that there was a large amount of fibrous tissue. In a few instances the section was crowded with leucocytes but no cause for this could be made out. No constant arrangement of the numerous small vessels could be discovered. The appearance of the section never bore any relationship to the cause of death.

I carefully examined all these ganglia with regard to their size, and found it varied as much as does that of the superior cervical, and that these variations were quite independent of the cause of death.

Sections were made of nine splanchnic nerves, and although some of them were attached to semilunar ganglia whose nerve-cells were degenerate, these nerves were always healthy.

Table D shows the appearances of the semilunar ganglia taken from eighteen mammals lower than man; the nerve-cells of all were examples of the normal type.

These results so agree with those arrived at on examination of the superior cervical ganglion, that we may probably conclude that, although the semilunar ganglia in the lower mammals and in young human beings are functionally active, in human adults their nerve-cells have degenerated and become functionally inactive, but the nerve fibres always retain their structure and function.

The superior cervical and semilunar are the two largest collateral ganglia in the body, therefore I suspect that the conclusions concerning them to which we have arrived, may be applied to all the collateral ganglia. As supporting evidence I would urge that degenerate nervous structures are known to exist in the human adult, for Gaskell (*Med. Chir. Trans.* Vol. LXXI. p. 361) has described a degenerate ganglion on the root of the third cranial nerve, and probably the medullary portion of the suprarenal organ should be regarded as an instance of degeneration, for it is of considerable size in the fœtus, but in the human adult is very small indeed in proportion to the whole body.

In order to see whether the lateral ganglia resemble the collateral I prepared the thoracic ganglia taken from twenty-four patients. Many sections of each were examined, and in most cases the ganglia were taken from both sides of the body. The results will be found in Table E, which consists of two parts, the first containing a description of the first thoracic ganglia, the second an account of thoracic ganglia below the first. The first thoracic ganglia were examined in 15 persons dying of various diseases. Those from the three youngest, viz. a child of five months, and two men aged respectively nineteen and twenty-three, showed perfectly normal nerve cells, which were large, distinct, nucleated, neither granular, nor pigmented, and the nerve cells were similar to these in sections taken from patients aged 27, 35, 36, 41, and 58. The nerve cells were slightly granular and pigmented, and therefore might probably be considered as showing some evidence of degeneration in patients whose ages at death were 24, 41, 57, 59, 60, 62, 63, but in none of these did the cells ever show the extremely granular and pigmented condition associated with atrophy, which is common in the superior cervical and semilunar ganglia of human adults. On comparison of the ages of the two sets of patients, it will be seen that those in whom the cells showed neither granular nor pigmentary change, were much younger than those in whom this condition was present. The average age of the former is 30, of the latter 52. As the inferior cervical ganglion is often blended with the first thoracic, the sections were always taken from the lower half.

Thoracic ganglia below the first were examined in nine persons. In six the nerve cells were large, well formed, and showed neither granular nor pigmentary change. In three this condition was present, but it never existed in the extreme degree so common in the superior cervical and semilunar ganglia. Unfortunately the ages of these three patients are not stated, but they died of maladies much more common in adults of middle or later life, than in younger persons.

I think this examination of lateral ganglia is sufficient to enable us to assert that they do not resemble collateral in the extreme degree of degeneration which these always present in human adults, but that in old age they show slight evidence of degeneration. This is supported by the fact that thoracic ganglia are much more constant in their size than are the superior cervical and semilunar.

In the same person similar thoracic ganglia from opposite sides of the body exactly resemble each other. The nerve fibres are always healthy in appearance. There is nothing noteworthy about the fibrous

stroma. The capsule of the cells is usually beautifully nucleated. The arrangement of the vessels is that there are a number of small ones which run parallel to the long axis of the ganglion. Occasionally some leucocytes may be seen in the meshes of the fibrous tissue. The sex and cause of death do not influence the appearance of the ganglion.

The conclusions which I would draw from these two papers are:—

Firstly; that in lower mammals and young human beings the collateral ganglia (if we may judge from the superior cervical and semilunar) are functionally active, but that in monkeys there are evidences of the commencing loss of their function which has completely disappeared in the human adult.

Secondly; that in man the function of the lateral ganglia is maintained well into adult life, and only begins to disappear in old age.

In the following tables the nerve cells are frequently described very briefly, but in those cases the description of sympathetic cells which I have given in the previous number of this *Journal* may be taken to apply.

TABLE A.

A list of Human Superior Cervical Ganglia, arranged according to the age at death, showing cause of death, and histological appearance.

1. Disease of knee. Age 2.
   Cells well formed and look healthy.
2. Diphtheria. Age 2½.
   Cells small but healthy looking, much connective tissue.
3. Diphtheria. Age 4.
   Cells large, numerous, distinct, and healthy looking.
4. Typhlitis. Age 10.
   Many of the cells are small but they look healthy.
5. Fractured skull. Age 21.
   Nerve cells are very small and shrunken, much connective tissue.
6. Suppurating kidney. Age 21.
   Some of the nerve cells are small, others are granular and pigmented.
7. Phthisis. Age 26.
   The cells are mostly granular, some are pigmented and some are shrunken, there is much connective tissue.

8. Phthisis. Age 27.
   The few cells that can be seen are small and degenerate.
9. Cystitis. Age 31.
   The cells are healthy looking.
10. Typhoid. Age 42.
   Some of the cells are granular, some are pigmented, there is much fibrous tissue.
11. Acute pneumonia. Age 41.
   Many of the cells are granular and shrunken.
12. Gangrene of the foot. Age 42.
   A few cells, and they are small, granular, and pigmented.
13. Phthisis. Age 42.
   The cells are small and indistinct, and there is much connective tissue.
14. Amputation of thigh. Age 42.
   Nothing but connective tissue, with a few small cells in the interstices.
15. Abdominal cancer. Age 48.
   Cells small, shrunken, granular, and pigmented.
16. Acute dermatitis. Age 50.
   There are very few cells, and they are small and degenerate.
17. Strangulated hernia. Age 55.
   There are but few cells, and they are small, atrophic, and granular, with much connective tissue.
18. Malignant disease of the pleura. Age 61.
   Only a few cells and they are degenerate, there is much connective tissue.
19. Acute pericarditis. Age 65.
   Some cells are granular, very few show any nucleus; there is much connective tissue.
20. Disease not stated. Age 70.
   A very few cells, and what there are present are granular and shrunken, there is much connective tissue.
21. Strangulated hernia. Age 70.
   Cells nothing but masses of pigment, there is much fibrous tissue.
22. Granular kidneys. Age 71.
   The cells are very small and nearly all of them are granular and pigmented.

TABLE B.

A list of Mammalian Superior Cervical Ganglia with histological appearances, probable age and cause of death.

A. CATARRHINA.

1. Rhesus Monkey (Macacus rhesus).
   About 8 years old; cause of death doubtful.
   Cells large, neither granular nor pigmented.
2. Bonnet Monkey (Macacus sinicus).
   Juvenile; cause of death rickets.
   Large well-formed nerve cells and fibres.
3. Large white nosed Monkey (Cercopithecus petaurista).
   About 3 years old; cause of death ascites.
   Plenty of large well formed nerve cells.
4. Anubis Baboon (Cynocephalus anubis).
   About 5 years old: cause of death periostitis.
   Several of the cells are granular and pigmented, the others are normal, the section is crowded with leucocytes.

B. CARNIVORA.

5. Lion (Felis leo).
   Still born.
   Cells very well formed and healthy looking.
6. Spotted Hyæna (Hyæna crocuta).
   Aged; cause of death doubtful.
   Cells large, distinct, and well formed.
7. Polar Bear (Ursus maritimus).
   Young, 9 months in Gardens; cause of death doubtful.
   The nerve cells are all healthy.
8. Polar Bear (Ursus maritimus).
   Young; cause of death abscess of jaw.
   All the nerve cells are distinct and healthy looking.
9. Polecat (Mustela putorius).
   Adult, 2 years in Gardens; body not examined.
   Cells typically normal.
10. Spectacled Bear (Ursus ornatus).
    Very old; cause of death renal calculus.
    Cells perfectly normal.
11. Otter (Lutra vulgaris).
    Juvenile; no cause for death found.
    Large distinct cells, well formed, healthy.

12. Crab-eating Raccoon (Procyon cancrivorus).
    Aged; cause of death goitre.
    Nerve cells perfectly normal.

C. UNGULATA.

13. Thar Goat (Capra jemlaica).
    7 years in Gardens; cause of death abscess of cheek.
    Section crowded with leucocytes, but the nerve cells are perfectly healthy.

14. Axis Deer (Cervus axis).
    Still born.
    Nerve cells perfectly normal.

15. Michies Tufted Deer (Elophodus Michianus).
    Aged; cause of death bronchitis.
    All the cells that are to be seen are perfectly normal.

16. Bosch Bok (Tragelaphus sylvaticus).
    6 years in the Gardens; no cause of death found.
    Cells perfectly normal.

17. White Collared Peccary (Dicotyles tajaçu).
    8 years old; cause of death not found.
    Cells normal.

18. White Lipped Peccary (Dicotyles labiatus).
    Aged; cause of death peritonitis.
    Large normal nerve cells.

19. Indian Muntjac (Cervulus muntjac).
    Adult, 5 years in Gardens; cause of death not found.
    Healthy nerve cells.

20. Moose (Alces machlis).
    Adult; cause of death tuberculosis of liver.

D. RODENTIA.

21. Hairy Rumped Agouti (Dasyprocta prymnolopha).
    Adult, 4½ years in Gardens; cause of death not found.
    Cells well formed, normal looking.

E. MARSUPIALIA.

22. Great Kangaroo (Macropus giganteus).
    Adult, 3 years in Gardens; body not examined.
    Cells large, well formed and healthy.

TABLE C.

A list of Human Semilunar Ganglia showing the microscopical appearances and cause of death.

1. Diabetes.
   There are but few cells and they are indistinct, much connective tissue.
2. Diabetes.
   Cells very small and shrunken.
3. Diabetes.
   Cells very small, pigmented, granular and degenerate, there is a large amount of fibrous tissue.
4. Diabetes.
   Fellow ganglion to the last, the same description applies.
5. Idiopathic Anæmia.
   Cells small, pigmented, shrunken, there is a large amount of fibrous tissue.
6. Anthrax.
   Many of the cells are obscure in their outline, granular and pigmented, the others are normal.
7. Cancer of the Bladder.
   Some of the cells are pigmented, others are large and well formed.
8. Aortic Disease.
   Exactly like the last.
9. Bronchopneumonia (child, aged 3).
   Cells are beautiful examples of normal nerve cells, with well marked processes, and distinct nuclei.
10. Cancer of Bladder.
    Cells very pigmented and granular, and there is much fibrous tissue.
11. Sarcoma of Pelvis.
    Cells are reduced to minute masses of granular pigment.
12. Cirrhosis of the Liver and granular Kidney.
    A few of the cells are pigmented, the rest are large and well defined, there is much connective tissue.
13. Chronic Bright's Disease.
    Large well formed healthy looking cells.
14. Cancer of the Bladder.
    The cells are nothing but minute masses of granular pigment.
15. Aortic Aneurism.
    Exactly like the last.

16. Ruptured Intestine.
    A few of the cells are small and pigmented, the rest are healthy looking.
17. Aortic Disease.
    The cells are minute masses of granular pigment.
18. Phthisis.
    The cells are large and well formed.
19. Tumour of the Brain.
    Some of the cells are pigmented and granular.
20. Myxœdema.
    The cells are well formed and healthy looking.
21. Chronic Bright's Disease.
    Cells are indistinct and there is no nucleus.
22. Chronic Bright's Disease (another case).
    Exactly like the last.
23. Purpura Hæmorrhagica.
    The section is crowded with leucocytes, but the nerve cells appear normal.
24. Diphtheria (child).
    The cells are perfectly normal nerve cells.
25. Scald (child).
    Exactly like the last.
26. Aortic and Mitral Disease.
    The cells are small and granular.
27. Cerebral Hæmorrhage.
    Like the last.
28. Disease not stated.
    The cells are nothing but minute masses of granular pigment.
29. Diabetes.
    Cells large, but very granular and pigmented, there are many leucocytes.
30. Diabetes.
    Some of the cells are granular.
31. Diabetes.
    Many small degenerated pigmented cells, but the section is crowded with leucocytes.
32. Phthisis.
    The cells are normal, but there are many leucocytes.
33. Sarcoma of Breast.
    The cells are normal.

TABLE D.

List of Semilunar Ganglia taken from the lower animals.

A. CATARRHINA.

1. Talapoin Monkey (Cercopithecus talapoin).
   The sections are almost entirely occupied by large well formed cells, each with a distinct nucleus but no processes. There is no trace of granulation, pigmentation, shrinking, or degeneration, all the cells have a distinct nucleus.
2. Bonnet Monkey (Macacus sinicus).
   The cells here are exactly like those in the last specimen. The ganglion has a rather thicker fibrous sheath than is usual.
3. Arabian Baboon (Cynocephalus hamadryas).
   The description of the first specimen applies exactly to this.
4. Green Monkey (Cercopithecus callitrichus).
   The cells are very numerous and very closely packed, but the description of those in the first specimen applies to these also.
5. Rhesus Monkey (Macacus rhesus).
   The same description holds for these specimens also, save that the section is crowded with leucocytes.
6. Malbruck Monkey (Cercopithecus cynosurus).
   These cells too are well formed, well nucleated, without any trace of granular or pigmentary change.
7. Macaque Monkey (Macacus cynomologus).
   The cells here are exactly like those in the previous described specimens.

B. CARNIVORA.

8. Philippine Paradoxure (Paradoxurus philippensis).
   The cells are large, well formed, perfectly healthy looking, well nucleated, without any granular or pigmentary change.
9. Suricata (Suricata tetradactyla).
   Cells like those previously described.
10. Common Genet (Genetta vulgaris).
    Cells like those previously described.
11. Virginian Fox (Canis virginianus).
    Cells like those previously described.
12. Grison (Galietis vittata).
    The cells are exactly like those previously described; the sections contain many leucocytes.
13. Common Cat (Felis cattus).
    The cells are exactly like those previously described.

14. Blotched Genet (Genetta bigrina).
The cells are exactly like those previously described.

C. RODENTIA.

15. Spotted Cavy (Cœlogenys paca).
The cells are exactly like those previously described.

16. Squirrel (Sciurus [sp. incert.]).
The cells are exactly like those previously described.

D. MARSUPIALIA.

17. Vulpine Phalanger (Phalangista vulpina).
Exactly like the specimens previously described.

18. Opossum (Didelphys virginianus).
The cells are exactly like those previously described; there are several leucocytes.

In all the above specimens the nerve fibres, blood vessels and supporting tissue were normal.

TABLE E.

List of Human Thoracic Ganglia.

A. FIRST THORACIC GANGLIA, with cause of death, sex, and age.

1. Cleft Palate, Bronchopneumonia. F., 5 months.
Ganglia small, and the nerve cells also, they fill their capsules, are well formed, nucleated, neither granular nor pigmented; nerve fibres healthy; the ganglia from the two sides are precisely alike.

2. Ruptured Kidney. M., 19.
Nerve cells large, fill their capsules, no pigmentary nor granular changes, many have a distinct nucleus, some have not; nerve fibres healthy; there are many blood vessels which run parallel to one another through the length of the ganglia; those from the two sides are precisely alike.

3. Cerebral and Hepatic Abscesses. M., 23.
Nerve cells are all large, completely fill their capsules, are neither granular nor pigmented; nerve fibres healthy; this ganglion has a particularly thick investment of fibrous tissue; the two are similar.

4. Psoas Abscess, Lardaceous Disease. M., 24.
Many of the nerve cells are small, but they all fill their capsules and several of them are a fair size; some have nuclei, some have not; several are granular and a few are pigmented, but I cannot make out that these changes bear any relationship to their size and none are smaller than their capsules; the section is full of small leucocyte-like cells; both ganglia are alike.

5. Puerperal Peritonitis. F., 27.

Nerve cells large, rounded, fill their capsules, neither granular nor pigmented, in most the nucleus is very distinct; the nuclei of the capsule show very well; the nerve fibres are healthy, and the two ganglia are alike.

6. Phthisis. F., 35.

Many nerve cells are rounded, and distinct without any granular or pigmentary change, in some the nucleus is not very evident; there are no processes; the two ganglia are quite alike.

7. Aneurism of the Aorta. M., 36.

These ganglia are exactly like the last, and the two ganglia are quite similar.

8. Phthisis and Bronchitis. F., 41.

The cells are well formed, distinctly nucleated, some have well marked processes, they fill their capsules, the nuclei of which are very evident; a few of the nerve cells show some granular pigment, but this never implicates the nucleus which always stands out prominently; there are no small atrophic cells; the nerve fibres are healthy; the two ganglia are quite alike.

9. Bronchitis and Emphysema. F., 41.

Most of the cells are beautiful examples of the rounded type, but a few have processes; the nucleus is distinct, and there is no granular change or pigmentation, they completely fill their capsules, which are well nucleated; the ganglion is very vascular, being pervaded by numbers of small vessels running parallel through its length; the two ganglia are alike.

10. Fractured Ribs, œdema of the Lungs. M., 57.

Most of the cells are granular and slightly pigmented, and a very few are abnormally small, but all fill their capsules, and none are without a distinct nucleus, nor are there any so pigmented, granular and shrunken as one often sees them in other ganglia; the fibres are healthy, and the two ganglia are alike.

11. Fractured femur, Bronchitis. M., 58.

The cells are large, rounded, and distinct, without any pigmentation, or granular degeneration; the nucleus shows well; there are many vessels all running through the ganglion parallel to its long axis; the nerve fibres are healthy; the two ganglia are alike.

12. Cancer of the Œsophagus. M., 59.

Most of the cells are large and well formed, sending distinct processes to the edge of the capsule; some of the large ones are slightly granular, and a few are small granular masses which only half fill their capsules. Although these cells resemble those often

seen in the superior cervical ganglion, they are not nearly so degenerate as is commonly the case in this ganglion; the nerve fibres are healthy; the two ganglia are alike.

13. Cerebral Vomiting. Nothing found at the autopsy. M., 60.
Most of the cells are large, neither granular nor pigmented, but some are small, slightly granular and a little pigmented, both varieties are well nucleated; the two ganglia are alike.

14. Cirrhosis of Liver. M., 62.
Numerous large, rounded, nucleated nerve cells without any processes, they fill their capsules which are well nucleated, a few show a little granular change at the edge; the two sections are alike.

15. Cancer of Œsophagus. M., 63.
The cells are large, rounded, well formed and distinct; many are granular and pigmented, but most that are in this condition are large and well nucleated, a few are small, but even in them there are large well formed nuclei; there are none of the minute masses of granular pigment so often met with in the superior cervical ganglia; the two ganglia are similar. In this as in all the preceding specimens the nerve fibres are healthy.

B. Thoracic Ganglia, below the first.

16. Acute Tuberculosis. Æt. 19.
Cells typically normal, large, well formed, neither granular nor pigmented, fill their capsules; there are many vessels and a few leucocytes.

17. Fractured Skull. Æt. 45.
This specimen is exactly like the last.

18. Cirrhosis of the Liver. Æt. 47.
The cells are large, well formed, and perfectly healthy; there are many leucocytes.

19. Diabetes.
Cells large, nucleated, neither granular nor pigmented.

20. Cause of death not stated.
Cells exactly as in the last specimen.

21. Diabetes.
Many of the nerve cells are small and granular with a good deal of pigmentation; there are several leucocytes; the ganglia on both sides are alike.

22. Fractured Thigh.
Nearly all the cells are large, well developed, fill their capsules and look like healthy nerve cells, but a few are faintly granular,

and some of these are rather smaller than the rest; the corresponding ganglia on opposite sides are similar.

23. Diabetes.

The cells are perfectly healthy looking, there are a great many vessels in the sections and many leucocytes; the ganglia on the two sides are alike.

24. Diabetes.

Most of the cells are large and distinctly nucleated, many are granular and slightly pigmented, but the nucleus always remains distinct; a few of the cells are small; there are many leucocytes; the ganglia from the two sides are similar.

THE INNERVATION OF THE RENAL BLOOD VESSELS[1]. By J. ROSE BRADFORD, M.B., D.Sc.; *George Henry Lewis* Student. Plates XXIII—XXVI.

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The work, of which the following is an account, was begun in 1887 at the suggestion of Dr Foster and Dr Gaskell, and to them my best thanks are due for much valuable advice and assistance on many occasions. It seemed desirable, in the light of Gaskell's[2] well-known work on the sympathetic system, to attempt to map out the origin, course and nature of the renal nerves more accurately than had hitherto been attempted. More particularly it was important to decide whether the vascular nerves were of two kinds, vaso-constrictor and vaso-dilator, or whether the latter nerves could not be demonstrated to exist. The research was carried out exclusively on dogs, inasmuch as this was the animal used by Gaskell in his anatomical researches on the sympathetic, and since my work consisted largely in going over his results from the stimulation point of view, it was obviously desirable to use the same animal. Further, the arrangement of the vaso-motor and viscero-motor nerves in the dog is very similar to what obtains in man. At any rate, this deduction is justifiable from the results obtained by anatomical methods in the dog and in man, and so we may conclude perhaps that the physiological nature of the nerves in the two is similar. If this conclusion is considered rather unjustifiable it will at least be conceded that the dog is an animal in which there will be at least a very great resemblance to man in regard to such a point as the general arrangement of the vaso-motor nerves; and certainly if renal vaso-dilator fibres can be demonstrated to exist in the dog, we may assume their existence in man. For these reasons, then, amongst others, all the following observations were made on the dog.

[1] An abstract of this paper has appeared in the *Proc. Roy. Soc.* Feb. 21, 1889.

[2] Gaskell, This *Journal*, Vol. VII.

The present paper will be divided into the following parts.

I. *The origin and course of the vaso-constrictor fibres.*
II. *The existence and course of the vaso-dilator fibres.*
III. *The phenomena following excitation of the splanchnic nerve.*
IV. *The phenomena following excitation of the peripheral end of the divided vagus.*
V. *The reflex phenomena of the renal vessels.*
VI. *Conclusions.*

Method.

Before describing the results of this research it will be necessary to describe in some detail the method employed.

The general blood-pressure and the volume of the kidney as registered by Roy's oncometer were recorded on the blackened surface of a kymograph, simultaneously with a time tracing and a lever marking the moment and duration of the nerve excitation. In this way the local and general effect of any given stimulation were both recorded, and in this manner numerous fallacies were avoided. Thus, for instance, it could be at once determined by mere inspection of the trace whether any given dilatation of the kidney was an active one, or due merely to a rise of general blood-pressure, in the causation of which the kidney vessels took no part.

In the earlier experiments Roy's oncograph was used as a recording instrument, but more lately, at Prof. Schäfer's suggestion, a piston recorder was used. Of course in this second method the changes in the volume of the kidney appear to be comparatively slight, as the effect is not in any way magnified. This apparent defect, however, really presents many advantages, as the oncograph is inconvenient where the changes in volume are not only sudden but also large.

It is very essential in these experiments, as they are necessarily of long duration, not to have any clotting in the carotid cannula, and hence a half-saturated solution of sodium sulphate was used in the pressure bottle.

The method of preparation and of stimulation of the nerves was as follows. The spinal canal was opened in the required region by removal of the neural arches, and the roots of the nerves excited after being previously divided. Sometimes the entire nerve outside the dura mater but inside the bony spinal canal was stimulated, after division of

the posterior root inside the dura mater. As a rule in any given experiment some three or occasionally four nerves were exposed, but it is desirable to expose as short a length of cord as possible, owing to the great shock produced by extensive exposure of this delicate structure. Inasmuch as the exposure of the nerves is the most difficult, and at the same time the most important part of the preparation, a few words may here be added as to the actual method employed. The essential points are the avoidance of shock as much as possible, and also the avoidance of hæmorrhage; the latter is comparatively easy, the former is best avoided by rapidity of operation, and also by not exposing a greater length of cord than is absolutely necessary. It is most important to have but little hæmorrhage, as otherwise the blood-pressure is sure to present great oscillations, due to imperfect Traube curves. For these reasons medium-sized fox-terrier dogs of about 25 lbs. weight were used, and having been deeply anæsthetized with chloroform or chloroform and morphia, the carotid artery and the kidney were prepared in the usual way[1]. An incision some three inches long was then made over the spines of the dorsal vertebræ in the region required, and the skin and subcutaneous fat cut through down to the spines. The vertebral aponeurosis is then cut through on each side of the spines. The mass of erector spinæ is now rapidly separated from the bones by a few touches of the knife, the bleeding being controlled partly by the fingers of the left hand compressing the muscles as they are separated, and partly by sponges pressed firmly into the wound by an assistant. After a few moments' firm pressure the sponges are taken out and all the bleeding points picked up with Spencer Wells' artery forceps. The muscles are then further separated from the vertebræ, until the transverse processes and ribs are quite clearly exposed at the bottom of the wound, the dorsal branches of the intercostal arteries being seized with Spencer Wells' forceps as soon as they are cut. If the operation be done rapidly as above described, there is far less bleeding than if the attempt be made to separate the muscles gradually and so avoid the vessels, which as a rule is impossible. All the larger vessels are tied or twisted, and the oozing is arrested by firm pressure with sponges for a few minutes. The bone forceps are then applied and the spines and neural arches wrenched off, the spinal cord enclosed in the dura mater being thus exposed. All bleeding being now arrested by torsion or ligature, the wound is packed with cotton wool, the kidney is then placed in the oncometer, and the cannula in the carotid connected with the mercurial

[1] Bradford and Phillips, This *Journal*, Vol. VIII. p. 117.

manometer. On now removing the cotton-wool it will be found that the cord is exposed at the bottom of a wound, which is practically dry, all the hæmorrhage having been stopped. It is absolutely essential to the success of these experiments not to have the cord lying at the bottom of a pool of blood, as then it is impossible to stimulate in such a manner as to avoid spreading of the current.

The dura mater is now opened and the posterior roots are then gently lifted up, ligatured, and divided distally to the ligature, the ligatured central end being thus ready for excitation. For efferent effects, the entire nerve outside the dura mater was excited, as just mentioned, after the previous section of the posterior root. As will be noticed more fully below this is practically equivalent to excitation of an anterior root, since no effects are seen to follow the stimulation of the peripheral end of a posterior root. Moreover, this method possesses the very great advantage of there not being nearly so much danger of spreading to the cord, as when an anterior root is excited inside the dura mater; however in many cases this latter mode of stimulation was used. In order to still further diminish the risk of confounding the results of efferent stimulation with those dependent on spreading of the current to the cord and the subsequent production of reflex effects, the spinal cord was divided transversely at the upper limit of the wound. In this manner, as proved by the results, practically all danger of spreading was eliminated.

The lower dorsal and upper lumbar nerves can be reached far more easily than the upper dorsal, and fortunately most of the kidney nerves run in these lower dorsal nerves. When observations were made on the splanchnic nerve this nerve was reached from behind by the resection of one or two of the lowest dorsal nerves.

The anæsthetic used was always chloroform, in many cases morphia as well was given, but it is necessary to be careful with the latter drug, as large doses of it apparently paralyse the activity of the vaso-motor nerves[1], so that excitation of the nerves produces no peripheral effects. Further, it is probable that the vaso-dilators are more readily affected by morphia than the vaso-constrictors. In doses which render the subsequent administration of the chloroform more easy morphia has but little effect in impairing the activity of these nerves, and as is so well known, it makes the chloroform narcosis much deeper, and at the same time more constant and hence safer. Finally, when everything was ready the animals were curarized, and artificial respiration (the air being blown over chloroform) maintained in the usual manner. In this paper the

[1] Compare Brunton, *Pharmacology and Therapeutics*, p. 771.

following anatomical nomenclature will be adopted, i.e. that the dog has "twenty dorso-lumbar vertebræ, of which thirteen are dorsal and seven lumbar[1]." This is no doubt the usual description; some authorities however have adopted a different one, owing to the statement that the number of ribs in the dog is somewhat variable. I cannot say that in the course of my observations I have met with any great variety, in fact I have found it quite exceptional for more than thirteen ribs to be present.

It is necessary to state this much about the method of nomenclature, since it is different to that adopted by Gaskell[2] in his observations on the sympathetic system. It is obvious that the 10th spinal nerve is in both cases the 2nd dorsal, and the 21st nerve is the 13th dorsal; but whereas with Gaskell the 25th nerve is the 2nd lumbar, according to the nomenclature adopted here the 23rd nerve will be the 2nd lumbar, and the 25th nerve the 4th lumbar. In other words, Gaskell recognises but five lumbar vertebræ instead of seven. According to his anatomical researches, the vaso-motor and viscero-motor fibres leave the spinal cord in the roots of the nerves extending from the 10th spinal to the 25th spinal nerve. That is to say, according to the nomenclature of the present paper, from the 2nd dorsal to the 4th lumbar. Finally, the method of stimulating the spinal nerve outside the dura mater, but before it leaves the spinal canal, would be considered by some open to criticism, in that the observations are then made on the entire nerve and not on the roots. There are, however, many advantages in this method. In the first place the roots themselves are exceedingly soft, and hence it is difficult to stimulate them many times in succession without at the same time damaging them, whereas the nerves outside the dura mater are dense and firm. But the great advantage is undoubtedly the avoidance of reflex effects due to spreading of the current to the cord. It is exceedingly difficult to stimulate roots in such a manner as to avoid spreading to the lateral column of the cord. If the wound is perfectly dry at the moment of excitation, owing to the heightened blood-pressure occurring at the moment the vaso-motor effect is produced, blood and fluid are liable to accumulate, leading infallibly to reflex spreading effects, notwithstanding the lifting up of the nerve and the use of shielded electrodes. On the other hand, if the nerve is stimulated outside the dura mater, more especially if it has been previously cut and ligatured, there is very much less danger of this happening. Of course the objection made is that this method does not differentiate the

[1] Huxley, *Anatomy of Vertebrated Animals*, p. 353.

[2] Gaskell, this *Journal*, Vol. VII.

fibres of the anterior from those of the posterior roots, but a number of observations were made on the effects of excitation of the peripheral end of divided posterior roots. It is with the peripheral end of anterior roots that the difficulty of avoiding spreading to the lateral column of the cord arises, it is not so difficult to avoid this with the peripheral end of the posterior roots. In no case, however, was any effect obtained when the peripheral end of a divided posterior root was excited after having been ligatured and lifted up out of the wound. This is quite in accord with Gaskell's[1] observations on the nervi erigentes in the rabbit; so the conclusion was arrived at that, at any rate as regards the kidney, there is no evidence in favour of the existence of efferent vaso-motor fibres in the posterior roots, such as Stricker[2] thinks to be the case for the nerves of the hind limb. It will be however more convenient to discuss Stricker's results at the end of this paper. Hence we may conclude that the excitation of the entire nerve cut and ligatured and excited outside the dura mater is practically equivalent to the stimulation of an anterior root. This is confirmed by the fact that the results of this mode of excitation are similar to those obtained by exciting anterior roots after the previous section of the spinal cord above the level of the nerve excited. In this manner all danger of reflex excitation was avoided.

For excitation a Du Bois Reymond coil with Helmholtz's modification was used, and the currents generally employed were perceptible to the tongue but not of greater intensity. For many experiments where the effects of the rate of excitation were investigated, a vibrating reed, still with Helmholtz's modification, was used, giving rates varying from 5 to 50 per second. In experiments on the existence of vaso-dilators a slower rate of excitation was used, i.e. 1 per second, and for this purpose an apparatus was used so that the make shocks were cut off and only the break ones were sent into the nerves.

Having thus described in some detail the method employed, we will now pass on to the consideration of the vaso-constrictor nerves, and the effects they produce on the kidney and general blood-pressure.

I. Origin and Nature of the Renal Vaso-constrictors. (Figs. 1 to 9.)

Entering the hilus of the kidney between the artery and vein there are, as is well known, numerous nerves, as a rule about a dozen. These

[1] Gaskell, *Proc. Phys. Soc.*, this *Journal*, Vol. VIII.

[2] Stricker, *Sitzb. der k. Acad. der Wissensck.* 1876, Wien.

nerves have long been known to contain vaso-constrictor fibres, since their stimulation is followed by both contraction of the kidney and diminution in the flow of urine. When these nerves are stimulated and the volume of the kidney is observed plethysmographically it will be seen that the renal contraction is sudden and considerable, but that the organ soon returns to its original volume. Similarly when the splanchnic nerve is excited the volume of the kidney diminishes rapidly and markedly, and synchronously with this renal contraction there is a great rise of general blood-pressure. Of course in this latter case the general rise of blood-pressure is due, not only to the renal contraction, but also to a widespread effect on the abdominal vessels. On exciting the lower dorsal nerves inside the spinal canal the same general facts are observed, i.e. a renal contraction and a rise of general blood-pressure, provided the posterior roots are previously divided, and care is taken to prevent spreading of the exciting current to the cord. Before entering into further details with regard to the course of these fibres, it is necessary to state that the exciting current must consist of either rapidly interrupted shocks, i.e. 50 per second, or at least a rate not slower than 5 per second. Even with this slow rate however some differences are to be detected, as will be mentioned further on. Hence unless otherwise stated it is to be understood that the rate of excitation was 50 per second.

The stimulation of the anterior roots, or of the entire nerve after division of the posterior root, or after section and ligature, is followed by contraction of the kidney and rise of general blood-pressure when any nerve from the 6th dorsal to the 2nd lumbar inclusive is the one selected and arranged for excitation. The stimulation of at any rate some of these nerves is followed by the above results in all cases, although in any given dog it may be found that this statement is not equally applicable to all the nerves just mentioned. Furthermore the excitation of the nerves higher up, e.g. the 4th or 5th dorsal, produces but slight effects on the blood-pressure; and when the uppermost nerves, e.g. the 2nd and 3rd are taken, of course the accelerator fibres of the heart are excited and a small rise of pressure due to these is then seen.

On the other hand, the 3rd lumbar has been found in many cases to produce no effects at all, and in other instances only a very slight rise of general blood-pressure. So that the 6th dorsal and the 2nd lumbar are practically the extreme limits of the nerves the excitation of which will produce any marked effect either on the kidney or on the general blood-pressure. No doubt it is rather surprising that the upper dorsal nerves produce comparatively so little effect on the general blood-pressure,

because even if the kidney and other abdominal viscera do not receive any vaso-motor fibres from these nerves, the head and neck and upper limbs do. Thus other observers[1] have shown that the vaso-motor fibres for the fore-leg run in the anterior roots, from the 2nd to the 5th dorsal, and the same is true of the fibres destined for the head and neck.

Hence one would no doubt expect the stimulation of these nerves to cause a rise of general blood-pressure, owing to the local constriction produced. The rise in general blood-pressure consequent on excitation[2] of the 3rd or 4th is measured by a very few millimetres of mercury, e.g. 10 or 15, whereas the 6th dorsal will frequently cause a rise of some 30 mm. Hg. When the accelerator fibres are stimulated, more especially in the 2nd and 3rd nerves, the rise of pressure is quite different in its course and character to that dependent on the peripheral contraction of systemic vessels. Hence, as we pass down the dorsal nerves we find that the upper ones produce but little effect on the general blood-pressure, the middle ones produce a greater effect, and the lower ones, i.e. from the 10th to the 13th, produce the greatest of all. Then when we come to the lumbar nerves the effects diminish, only much more suddenly, so that the 1st lumbar does not cause such a rise of general blood-pressure as the 13th dorsal, the 2nd lumbar causes still less, and the 3rd lumbar frequently produces scarcely any result at all. We must not perhaps hastily conclude from this that the 3rd lumbar contains no vaso-motor fibres, since it may very well be the same with this nerve as with the uppermost dorsal nerves, where, as mentioned above, no very marked general effect is seen to follow their excitation, and yet they undoubtedly contain vaso-motor fibres for the upper limbs, head and neck, &c. No doubt a tolerably large effect must be produced locally in order for a marked effect to be seen on the general blood-pressure, but at the same time it is probable that the vaso-motor mechanism of the visceral vessels is better developed than that innervating the skeletal muscles. As mentioned above I propose to leave to a subsequent paper the discussion of the vaso-motor fibres in the 2nd, 3rd, 4th, and 5th dorsal nerves, since this would entail the discussion of the nerves supplying the pulmonary vessels,—work I have been engaged in jointly with Mr Dean[3]. In the present paper, therefore, we will only consider the facts concerning the

[1] Dastre et Morat, *Système Nerveux Vaso-Moteur*, p. 130, Paris, 1884.

[2] In a future communication by my friend Mr Dean and myself we hope to enter into this question more fully.

[3] Bradford and Dean, "Innervation of Pulmonary Vessels," *Proc. Roy. Soc.*, Feb. 1889.

nerves from the 6th dorsal to the 2nd lumbar, or 15th dorsal of Gaskell.

Now although the excitation of all these nerves produces contraction of the kidney and rise of blood-pressure, yet on the whole the lower nerves are more efficacious than the upper. Just as we found above that the greatest effects on the general blood-pressure were obtained when the lower nerves are excited, so also the greatest contraction of the kidney is caused by stimulation of the 11th, 12th and 13th dorsal nerves. Thus the 5th dorsal generally causes a rise of blood-pressure equal to about 20 mm. Hg., the 6th to the 10th give a rise of some 20 to 50 mm., and the lowest dorsal frequently produces a rise of as much as 60 mm. Hg. The kidney contraction follows the same order; the contraction produced by the 12th or 13th nerves frequently being twice or three times as great as that caused by the 6th or 7th nerves. So that although all the nerves from the 6th dorsal to the 2nd lumbar may produce an effect on the kidney, yet the nerve fibres passing out from the cord through the anterior roots, from the 10th to the 13th dorsal, must be looked upon as containing more especially the fibres destined for the renal vessels. It is no doubt surprising at first sight, to find so little differentiation between the nerve supply of the kidney vessels and that of the vessels of the other abdominal viscera. For it is clear from the above description that the kidney receives vaso-motor fibres from all the nerve roots supplying the abdominal viscera. That is to say, that apparently from the 6th dorsal[1] to the 2nd lumbar the anterior roots of the spinal nerves contain efferent vaso-motor fibres for the vessels of the abdominal viscera, and inasmuch as all these nerves produce effects on the kidney there can be no great differentiation of the renal vaso-motor fibres from the fibres for the other abdominal vessels. In other words, the area from which the kidney vessels are supplied is a very extensive one, and apparently co-extensive with that supplying the abdominal vessels. It may be, however, that other abdominal organs, e.g. the spleen, receive their nerves from a more restricted zone, but at present I have not made any observations on the vascular supply of this organ. Now there are at least two explanations possible of the fact of the kidney receiving such an extensive nerve supply; one may be called, for the sake of simplicity, the morphological, the other the physiological. On morphological grounds the renal nerves might be expected to be derived

[1] The 4th and 5th dorsal nerves also probably contain a few fibres for the abdominal viscera. In other words the upper limit, like the lower, is not a sudden one, but rather a gradual one.

from an extended spinal area, since the kidney is not only a segmental organ, but it is an organ which extends far forward into the anterior segments. Hence there is nothing extraordinary in its deriving nerves from a segment so anterior as the 6th dorsal. This is more especially the case since the heart is known to receive its fibres from the 2nd to the 5th dorsal, and, as mentioned above, the vaso-motor fibres for the head, neck and fore limbs have also been shown to run in these nerve roots. On a morphological basis, then, we could quite understand that the kidney vessels should be innervated from the 6th dorsal to the 2nd lumbar. Probably this explanation is more correct than the second possible explanation, i.e. the one called for brevity's sake the physiological. We must remember that the vascular and visceral nerve fibres emerging from the cord by the anterior roots are connected to one another by means of a series of ganglia and commissures, forming the so-called sympathetic system. However true the great principle may be that the sympathetic system is essentially a transverse system metamerically repeated, and not a longitudinal system, yet the longitudinal commissures must place the different segments of this visceral nervous system in very close union. Hence it is at least possible that when a single anterior root is excited, the impulses so generated may affect more than one segment of this sympathetic system, and might under certain conditions perhaps spread to the visceral system generally, and so affect practically all the abdominal vessels. No doubt this does occur to a certain limited extent, since if a single anterior root be excited, a comparatively large area of blood vessels are affected, as is shown by the height to which the blood-pressure rises. To what extent the ganglia of the lateral and of the collateral chains act as centres for what may be called the diffusion of impulses is not known, but inasmuch as stimulation of some of these nerves may produce effect on the general blood-pressure as great as those following excitation of the splanchnic, it is clear that some such diffusion must occur. If, however, this diffusion occurs to a large extent, it is difficult to estimate exactly the significance of the effects produced by the excitation of any given anterior root, since any effect produced on the renal vessels might be due to one of two reasons. In the first place, the nerve in question might contain fibres going to the kidney, or, secondly, it might contain no such fibres, but only nerves passing to a mechanism of nerve cells and fibres in this sympathetic system, which mechanism innervated the kidney amongst the other abdominal viscera. If this latter interpretation were correct, any attempt to map out the cause of the renal nerves would be hopeless,

inasmuch as the fibres in the anterior roots would simply be commissures between the spinal cord and this great visceral system, innervating all the blood vessels of the abdomen, the renal vessels amongst the others. In other words, the sympathetic system would again be considered to be a more or less independent nervous system, instead of a mere dependence of the cerebro-spinal system. This view can, I think, be refuted not only on theoretical and morphological grounds, but also on the consideration of direct experimental facts. If the effects observed on the kidney and blood-pressure on excitation of nerves from the 6th dorsal to the 2nd lumbar were simply due to the stimulus causing a number of centres in the sympathetic to discharge, and so affect the vessels of the abdominal vessels more or less indiscriminately, we should expect equal results to follow the excitation of each and every nerve from the highest to the lowest. That is to say, not only ought the 8th dorsal to produce the same effects on the blood-pressure as the 13th dorsal, but the amount of the contraction of the kidney ought to vary pari passu with the height to which the general blood-pressure rises. The higher the blood-pressure the greater ought the renal contraction to be, and vice versa. This, however, is by no means the case, as mentioned before, the lower nerves produce larger effects both on the general blood-pressure and on the kidney. Further, it is not at all uncommon for a nerve to produce a large renal contraction and but a slight effect on the general blood-pressure, and for another nerve to cause great contraction of the kidney with a comparatively small rise in the general arterial tension. Hence, although the renal nerves are distributed over a large area of the spinal cord, and although the stimulation of any given spinal nerve affects other viscera than the kidney, yet nevertheless we must conclude from the above facts that the correct explanation of the extensive origin of the renal nerves is the morphological one. That is to say, that the kidney receives nerves, even in the dog, from a very large number of spinal segments, and that this spinal area is probably coextensive with that supplying the other abdominal vessels, because the kidney is morphologically speaking an organ of such great length.

We will now pass on to the consideration of the individual nerves, it being understood that the uppermost dorsal nerves, i.e. down to the 6th, will be considered in a subsequent paper, and hence it is only necessary to say here that the results of the stimulation of these nerves on the general blood-pressure are but small.

6th Dorsal Nerve.

Stimulation of this nerve outside the dura mater with the posterior root divided and with a quick rate of excitation leads to a rise of general blood-pressure rather slight in amount and slow in its course. The effect on the kidney is very small, and in many cases practically no contraction of the renal vessels is to be detected. The rise in general blood-pressure is quite small. Thus in one instance it was not more than 16 mm. Hg. whereas in the same animal the 7th dorsal produced a rise of 30 mm. Hg. This nerve, then, as a rule contains comparatively few vaso-motor fibres, and apparently but a very few of those present are destined for the kidney vessels. In a few instances the excitation of this nerve has caused a slight expansion of the kidney. This expansion is small in amount, but inasmuch as the kidney returns to its previous volume afterwards it is obviously due to the excitation. Since this nerve does not produce any marked renal contraction, and since occasionally this expansion is seen, it might be concluded that this nerve contained vaso-dilator fibres in more abundance than vaso-constrictor fibres. It is more probable, however, that in the majority of cases the 6th dorsal contains but few nerve fibres for the vessels of the kidney, and that the expansion described above is really due to the nerve containing vaso-motor fibres for other regions, probably some other abdominal vessels. It is clear that if this nerve supplied vaso-motor fibres to some other organ and really none to the kidney, the contraction of the vessels caused by the excitation would heighten the general blood-pressure. This increased arterial tension would tend to cause dilatation of those vessels not immediately concerned in the production of this increased tension. Hence the kidney vessels would dilate passively. Although the kidney sometimes expands on excitation of the 6th dorsal, this effect is probably not due to this nerve supplying the renal vessels with vaso-dilator fibres, and so producing an active dilatation, but rather to a passive dilatation consequent on increased arterial tension, due to peripheral constriction in other parts. Not only is this true for the case where the kidney receives no vaso-motor fibres from this nerve, but it would also be true if the nerve contained a very few fibres for the kidney, and a large number for other abdominal vessels. In this latter instance, it is possible if the part taken by the renal vessels in raising the blood-pressure was but small, its effects on the kidney would be overpowered by the contraction elsewhere, and so the renal

vessels might dilate slightly. In other words, no slight renal dilatation can be taken as evidence of the existence of vaso-dilator nerves, if this dilatation be accompanied by a rise of general blood-pressure, even if this rise be only small in amount. The effects, then, of excitation of the peripheral end of the 6th dorsal nerve are small, both on the general blood-pressure and on the kidney vessels. The latter, indeed, are often affected purely in a passive manner.

We may thus conclude that this nerve contains but few vaso-motor fibres relatively to the lower nerves, and that frequently scarcely any of these fibres are distributed to the kidney vessels. In the majority of cases however the kidney vessels receive some fibres from this nerve. This nerve is a good example in favour of the argument advanced above, with regard to the morphological arrangement of the nerves.

7th Dorsal Nerve.

This nerve, when prepared and excited in the usual way, gives much larger and more definite results than the 6th dorsal. Thus the rise of blood-pressure may be as much as 30 mm. Hg. with the same strength of stimulus that caused only a rise of 16 mm. Hg. with the 6th nerve. The blood-pressure rises slowly to its maximum, and then declines more slowly, so that frequently it is only some minutes after the cessation of the excitation that it regains its previous level. The effects on the kidney are also quite as marked. After a variable but short latent period the volume of the kidney diminishes considerably. The contraction is not very rapid, as frequently 10″ to 20″ elapses before the greatest diminution is reached, which may be after the excitation has ceased. Then the kidney begins slowly to expand, but as a rule it does not quite regain its former volume, or at any rate it does so very gradually. Hence there is a more or less persistent after-effect, so that at any rate for some little time the kidney is rather smaller than it was previously to the excitation. Not unfrequently the return of the kidney to its previous volume is not quite so simple, and its course is interrupted by what may be called a "secondary contraction." That is to say, that after the kidney has commenced to reexpand, and therefore long after the excitation has ceased, its vessels again contract, so that its volume diminishes and then the organ slowly returns to its previous size. As a rule this secondary contraction is quite small in amount, and no corresponding effect is seen on the blood-pressure curve. When however the kidney effect is a large one, a slight rise and fall of the blood-

pressure occurs with it, and then both the kidney and blood-pressure trace greatly resemble a Traube curve. In rare instances instead of one such secondary contraction of the kidney occurring, there may be two, and then of course the resemblance to a Traube curve is greater still. These spontaneous variations in the size of the renal vessels occurring after excitation of a nerve are not restricted to the 7th dorsal nerve. They may occur after the stimulation of any nerve, but they are mentioned here, as Fig. 1, Plate XXIII., gives a good idea of them. Perhaps the phenomenon is allied to the fact that occasionally the heart muscle will give a series of beats in response to a single excitation. So here the arterial muscle, after being thrown into a state of contraction by an artificial excitation, undergoes subsequently one or two spontaneous contractions of less magnitude. In both cases the rhythmical activity of the muscle is called into play, but inasmuch as this is better developed in the case of the heart muscle, this series of responses to a single excitation is not often seen in the case of arterial muscle.

With the 7th dorsal the fact previously pointed out, that the amount of the renal contraction does not vary pari passu with the rise of blood-pressure, is frequently observed. Thus on one occasion the same stimulus in the same animal caused every time practically the same increase of arterial tension, i.e. a rise of pressure of about 30 mm. Hg. In one case the kidney contracted to such an extent that the lever of the oncograph went off the paper. Whereas in a second instance the volume of the kidney was not appreciably altered in amount. In this latter case from the rise of blood-pressure it was clear that the excitation had been effectual, yet from the absence of any marked effect on the kidney it might easily have been thought that really no stimulation had occurred. No doubt this result is partly to be explained on the ground that the renal nerves are mixed, vaso-dilator and vaso-constrictor fibres, as will be explained more fully below, but still it is certainly remarkable that no diminution in the volume of the kidney should have been produced, along with the contraction occurring in the other abdominal vessels. At the same time although the volume was not diminished, the character of the trace was markedly altered, so that the cardiac pulsations were not nearly so evident. In some cases they have been almost obliterated. Is it possible that the vessels had become more or less rigid tubes, but that no great alteration in their actual calibre had taken place? No doubt it may seem fanciful to suppose that the arteries of the kidney could be affected by the excitation, and yet no change of volume of the kidney occur. If however

the character of the trace becomes altered when no such corresponding change occurs in the general blood-pressure, it is clear that the excitation has produced some local change in the particular organ investigated. It will be seen by inspection of Fig. 2, Plate XXIII., that there is a marked alteration in the character of the kidney trace, whereas there is no such alteration in the blood-pressure curve. Now if this figure be compared with others, i.e. Fig. 3, it will be seen that in the latter case also there is this curious alteration in the character of the kidney tracing, but here it is accompanied by a fairly well-marked contraction of the kidney, so that the stimulation has produced a distinctly twofold effect. Now it is clear that the diminution in size of the kidney is due to contraction of its vessels, and it is equally clear that the other effect is produced by some alteration of the vascular wall, which, whatever its nature, does not prevent the entry of blood into the kidney. Now if the vessels were to become more or less rigid without their calibre being markedly affected it is conceivable that an alteration in the character of the curve would be produced, and yet owing to their diameter remaining the same there would not be any increased impediment to the entry of blood. Hence the kidney would not receive a diminished supply of blood, and hence there would be no diminution in its volume, in fact with a heightened blood-pressure there might be an actual increased rate of flow through the renal vessels. If this interpretation is correct we must assume that a nerve is capable of causing a kind of tonic contraction of the arterial muscle, without materially affecting its calibre. No doubt this interpretation will not be readily accepted, but it is clear from the inspection of these and many other such tracings that a nerve is capable of producing more than one effect on the vessels. Further, it may very well be that the reason there is no diminution in the size of the vessels, and hence of the organ, is that owing to the high blood-pressure the muscular coat of the artery cannot overcome this pressure sufficiently to narrow the bore of the vessel. Hence the arterial wall only becomes rather more rigid than it was previously. After all, by accepting this explanation we should only be bringing the arterial muscle more into harmony with what is known of cardiac muscle, which, as has been ascertained, possesses a marked property of tonic contraction more or less independent of its rhythmical activity. This is often seen of course in the ventricle of the frog, and also, for the matter of that, in the dog, and it is a condition which is easily intensified by certain drugs. Thus digitalis, for instance, not only markedly affects the rhythm of the heart,

but it also causes the production or intensification of this tonic contraction, so that the rhythmical beats are superimposed on the gradually and persistently contracting ventricle.

No doubt in the arterial system this persistent contraction or tonus is usually closely connected with the active or passive alterations in the calibre of the vessels, but, as just shown, these two effects can be disassociated from one another, so that the excitation of a nerve can cause the arteries to assume persistently their previous mean diameter. Further, it must be remembered that in the particular instance from which these figures are taken these different effects were obtained from the same nerve with the same excitation, and as far as can be determined with the surrounding conditions the same.

The most important deduction however is, that apparently the stimulation of a vaso-constrictor nerve can materially affect the nature of the contraction of an artery without greatly affecting the amount of blood distributed to that organ. Hence if the blood-pressure be raised at the same time it is conceivable that an actual increased flow of blood might be produced. This point is no doubt of particular importance in the case of the kidney, where the secretion is at any rate closely connected with the rate of blood-flow. In the present paper, however, we are only concerned with the phenomena of the renal circulation. The question of the exact relation of secretion to circulation must be left for a future research.

8th Dorsal Nerve.

The effects produced on the kidney by the excitation of this nerve are much more marked than in the case of either the 6th or the 7th nerve. In the great majority of instances the contraction of the kidney is very marked, the recording lever falling abruptly, and the tracing exhibiting all the characters described above. With this nerve the point mentioned above, that the amount of the renal contraction is not always proportional to the rise of blood-pressure, is especially well seen. Thus in one case the 7th dorsal gave a rise of blood-pressure equal to 30 mm. Hg. and but a small contraction of the kidney. The 8th nerve in the same animal and with the same excitation caused a scarcely appreciable rise of blood-pressure, but the renal contraction was very marked and persistent. This nerve contained a considerable number of vaso-motor fibres for the kidney, and but few for the other abdominal vessels, whereas with the 7th nerve the relative proportions were more

or less reversed. This observation also shows how a comparatively large contraction of the kidney will produce little or no effect on the general blood-pressure. That is to say, that notwithstanding the extreme vascularity of the kidney, it requires a larger area than that of the renal vessels to produce by variations in the calibre of the vessels any very marked effect on the general blood-pressure. In one instance (Fig. 4, Plate XXIII.) expansion of the kidney unaccompanied by any alteration in the general blood-pressure was observed on excitation of this nerve. In this one case the effect was obtained every time the nerve was stimulated, and the greatest precautions were observed to eliminate any error due to spreading of the exciting current to the cord, and so producing reflex effects. The effect was small in amount but still perfectly distinct, and inasmuch as there was no variation in the general blood-pressure, it was probably due to a true vaso-dilator excitation. This point will be discussed below, but it is to be noted that the usual quick rate of excitation was used. This is one of the very few instances in which a dilatation of the kidney, unaccompanied by a rise of blood-pressure has been observed when quick rates of excitation have been employed. In this respect this result stands in marked contrast to that described above, as occurring sometimes with the 6th dorsal, where the renal dilatation was accompanied by a rise of blood-pressure. In the latter case the renal effect was undoubtedly a passive one. In the former, i.e. with the 8th nerve, the effect was probably as purely an active dilatation.

In no other case did the 8th nerve yield a pure dilatation of the kidney, and it is most exceptional for any nerve, with the usual method of stimulation, to yield such a result. It is possible that in this exceptional case, the 8th nerve may not have caused an active dilatation of the kidney, but it is extremely improbable that such an expansion could have been produced by contraction occurring elsewhere, insufficient to show its presence by raising the blood-pressure. Hence, inasmuch as the vaso-dilators can be demonstrated to exist by other and conclusive methods, as will be shown below, it is more reasonable to assume that in this case the 8th nerve contained vaso-dilators for the kidney, or at any rate that the nerve contained more vaso-dilator than vaso-constrictor fibres.

9th Dorsal Nerve.

Whereas with the nerves hitherto considered, all the effects are comparatively small and also to a certain extent variable, this is no longer the case when we reach the 9th nerve. In no case has the excitation of this nerve failed to produce an effect on the kidney, and in no instance, with the usual method of excitation, has any renal expansion been observed. So that in all cases the stimulation of this nerve has produced a contraction of the kidney generally of a very marked character. This contraction has all the characters described above, that is to say, it has a short latent period, and although its onset is sudden, its course is rather slow, and there is nearly always a persistent after-effect, the kidney not returning quite to its original volume. Further, as before, this renal contraction is accompanied by a rise of general blood-pressure, fairly marked in amount. In individual cases there is some variety both in the amount of the renal contraction and of the accompanying rise of blood-pressure, but these variations are comparatively insignificant as compared with those occurring with such nerves as the 6th and 7th dorsal. No doubt in this case also they have the same general significance as with the upper nerves. There are no doubt considerable variations in the course of the vaso-motor fibres, and sometimes in a given nerve there will be a preponderance of fibres for the kidney, sometimes there will be a greater relative number of fibres for the other abdominal vessels, thus tending in the latter case to a greater general effect, and a smaller renal effect. In some instances this contraction of the kidney is followed by what has been termed above a "secondary contraction," that is to say, that after the excitation has ceased and the kidney is expanding slowly, it suddenly undergoes a temporary contraction. This "secondary contraction" as seen in Fig. 6, Plate XXIII., generally occurs when the kidney has only partially returned to its previous volume, and thus a long time after the excitation has ceased, in this case 20″ after. This "secondary contraction" is slow in its course, and as a rule leaves a persistent after-effect, so that the kidney does not return quite to its previous volume.

This renal effect is frequently small, so that no corresponding effect is seen on the blood-pressure curve; at other times, as will be mentioned below, it is accompanied by a slight fall of general blood-pressure. This "contraction" of the kidney occurs at a time when the general arterial tension is falling, i.e. returning to the level at which it was being main-

tained previously to the excitation. Hence this shrinking of the kidney might be considered to be due to this fall of blood-pressure. This however cannot be the case, since at the time that this "secondary contraction" begins the kidney although dilating is yet more contracted than previously to the stimulation. Hence the blood-pressure is still higher than the normal, and the return of this to the normal is of course associated with dilatation of the previously contracted vessels. It is this dilatation of the renal vessels that is suddenly interrupted by this "secondary contraction." As mentioned above when this "contraction" is marked in amount it is accompanied by a slight fall of aortic pressure, e.g. some 5 mm. Hg., and this fall of blood-pressure is slow and gradual, just like the slow and gradual renal "contraction." It is this slow and gradual character of the kidney contraction that distinguishes it at once from the "secondary contraction," described as occurring occasionally and as producing results, in some respects, like Traube curves. This was described with the 7th nerve, and is seen in Fig. 1.

If this be compared with Fig. 6 illustrating the effect we are now describing as occurring with the 9th and other nerves, the difference between the two phenomena will be at once evident. That occurring with the 7th nerve is seen to be rapid, and soon the kidney dilates again; the heart-beats are also not so evident on the tracing, and in fact everything points to this result being a true *contraction*, i.e. an active process. On the other hand, with the 9th nerve this "contraction" is slow, the organ does not regain its former volume readily, if at all, and the heart-beats are not at all obscured; they are perhaps more obvious. Hence the character of the trace points to the phenomenon not being due to an active contraction. So that we shall term it not a "secondary contraction" but a "secondary shrinking," in order to distinguish these two results. Now, as just mentioned, this "secondary shrinking" if at all marked is accompanied by a slight fall of general blood-pressure and the two phenomena are undoubtedly closely connected; in fact, the fall of pressure is the actual result of the "secondary shrinking." That this is so is shown by the following considerations. It can be seen by inspection of the tracings that the fall of aortic pressure is not due to any cardiac effect, and hence it must be caused by some peripheral dilatation. Now if this dilatation occurred in some other area than that of the kidney it would obviously cause a diminution in the bulk of this organ, since the blood destined for the renal vessels would be diverted into other channels. Now the same is true, even if the renal vessels share in the dilatation, provided the total area affected is a large one, so that the

kidney vessels form but a small fraction of it, and provided also that the renal vessels are less affected than those of the larger area. Under these circumstances the kidney would shrink, owing to the fall of blood-pressure produced by the visceral dilatation, secondarily affecting the kidney, the vessels of this organ not having much share in the production of the fall of blood-pressure. We may express the same result in other words by saying, that just as the contraction of the kidney vessels plays but a small share in the causation of the general rise of blood-pressure, so the fall of pressure is mostly dependent on the dilatation of vessels in other areas than the renal one, although no doubt the kidney vessels also dilate. It will no doubt be expected that the kidney should undergo expansion in the latter case, corresponding to the contraction in the former. This however is erroneous, the actual expansion of the kidney depends not only on whether its vessels dilate, but also as mentioned above, on whether the dilatation is confined to the kidney or whether it affects this organ amongst the other abdominal viscera. This argument will appear more conclusive when the effects of the excitation of vaso-dilators, and also the results following such reflex excitation as the depressor, have been considered. Only one other point will be commented on here. It will be seen that this passive or secondary "shrinking" occurs at a time when the renal vessels are still somewhat contracted, as a result of the stimulation. This is seen by the fact that the kidney is not only smaller than previously to the excitation, but also by the fact that the general blood-pressure is often slightly higher than normal. Hence this contraction of the renal and other vessels gives way, and the blood-pressure falls, owing to this peripheral dilatation.

It must be remembered that this "secondary shrinking" after the cessation of the excitation is a result that is only observed in a minority of cases. When, however, this result is seen, the effects of the nerve excitation are really threefold, both on the kidney and on the general blood-pressure. Thus during the excitation the renal or other vessels undergo great contraction, and this contraction is of course accompanied by a great rise of blood-pressure. Then the renal and other vessels dilate, and the blood-pressure falls towards the normal, remaining however a little above it; this slightly heightened arterial tension is associated with some remaining constriction of the renal and other vessels. That is to say, that the vessels do not dilate to their previous size, but remain somewhat persistently contracted. Finally, in some cases this persistent effect is followed by an after-effect of a fall of

blood-pressure, due to a peripheral dilatation, shown in the case of the kidney, however, by a passive or secondary shrinking. The first two of the above effects are constant, the third, i.e. the dilatation, is not. The relation of this effect to what Dastre and Morat have termed "surdilatation," and also the question as to its exact cause, i.e. whether it is an exhaustion effect or whether it is due to the excitation of vaso-dilator fibres, will be discussed below.

10*th Dorsal Nerve.*

This nerve, prepared and excited in the usual manner, gives results essentially similar to those just described for the 8th and 9th dorsal. But just as the effects of the 9th nerve are better marked than those of the 8th, so the results following excitation of the 10th are larger in amount than those seen with the 9th nerve. Thus, in one instance, the rise of general blood-pressure was as much as 50 mm. Hg., and this is by no means an extreme case. Further, the renal contraction is always well marked and of a persistent character, the kidney but rarely returning completely to its former volume. In a few instances excitation of this nerve has been followed by a slight renal expansion, accompanied, however, by a small rise of blood-pressure, and in a very few cases the renal expansion has occurred alone, i.e. without any rise of blood-pressure. It is possible, although not probable, that these results were due to the excitation of vaso-dilator fibres, not to slight spreading of the exciting current to the cord. Much better evidence, however, than this will be adduced in favour of the existence of vaso-dilators; so although this occasional slight expansion with ordinary excitation may be due to the stimulation of vaso-dilator fibres, yet such facts as these would not be of much value in attempting to prove their existence.

The 10th nerve, however, affords an instance of another occasional result with ordinary quick rates of excitation, and this is well seen in Fig, 5. Plate XXIII. Here although the usual effect is produced on the general blood-pressure, i.e. a marked rise, the kidney effect is a mixed one. The contraction is preceded by a slight expansion, which, however, is only slight from its being interrupted in its course by the subsequent contraction. Now this initial expansion is due to one of two things, either the kidney vessels were not affected by the excitation at first, i.e. their latent period was longer than that of the vessels of the rest of the abdomen, or else the nerve stimulated contained both vaso-

dilator and vaso-constrictor fibres, and the latter effect preponderated over the former. With the former explanation the renal expansion is a passive one, owing to vascular constriction produced elsewhere. On the latter assumption the renal expansion is a more or less active one. In favour of the passive expansion is the fact that the rise of general blood-pressure begins synchronously with this expansion, and therefore long before the renal contraction, so that the excitation must have produced a vascular constriction in some other areas. It is equally clear that this particular nerve contained renal vaso-constrictors, since a renal contraction was ultimately produced. If the nerve contained only vaso-constrictor fibres, it is not clear, to say the least, why there should be such a difference in the latent period of those distributed to the kidney, and those going to the other abdominal vessels. Hence it is probably more accurate to consider the mixed result as due to the excitation of a mixed nerve, containing a few vaso-dilator fibres in addition to the large number of vaso-constrictor fibres. This conclusion would not be justifiable were it not that observations will be cited below actually proving the existence of vaso-dilator fibres in this and other nerves distributed to the kidney. Whichever view we accept this experiment shows that there was at least some differentiation in the results following the nerve excitation, and that all the vessels supplied by this nerve were not equally and similarly affected by its excitation. It is to be remembered, of course, that mixed effects on the kidney, like those just described, were not often observed. Much more commonly the result is a pure renal contraction, but these occasional results are valuable in affording evidence of variations in the course of the nerve fibres, and also as demonstrating to a certain extent the mixed character of the nerves.

Besides the occasional dilatation just described, and also the "secondary shrinking" due to a fall of blood-pressure, itself caused by a vascular dilatation, the initial renal contraction is sometimes followed by a "secondary contraction" like that described above with the 7th nerve. An example of this is seen in Fig. 7, Plate XXIV. Here this "secondary contraction" is undoubtedly of an active character, although from its being small in amount it does not produce any observable effect on the general blood-pressure. This contraction is soon over, but the subsequent expansion is again frequently followed by a second contraction, also of an active character, so that the kidney trace acquires the character of a Traube curve. In these instances then the initial contraction of the renal vessels has been followed by subsequent

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contractions, more or less rhythmical in their character, inasmuch as at least two are seen after the cessation of the excitation, and as mentioned above when describing the same effects with the 7th nerve, it is probable that they are due to the excitation of the rhythmical activity of the arterial muscle. Thus in experiments involving either direct or reflex stimulation of vaso-motor fibres, it is not uncommon for Traube curves to make their appearance when the excitation has ceased. Of course it is to be understood that no such curves were visible prior to the stimulation. Surely this tendency of the muscular wall of the vessels to undergo rhythmical contraction, after having been thrown by the stimulus into a state of persistent contraction, is allied, as mentioned above, to a series of cardiac beats following a single excitation.

11*th and* 12*th Dorsal Nerves.*

These two nerves may be considered together, since the effects following their excitation are not only essentially similar but they are also very nearly equal. These nerves produce greater effects on the kidney than any hitherto considered. The effect on the kidney is most frequently contraction pure and simple. The mixed effects described above are seen much more frequently with the higher nerves, and but rarely with these. On the other hand, although the renal contraction is very marked and persistent in character, the rise of general blood-pressure is not markedly greater than with the middle nerves which produce a smaller kidney effect. These nerves afford evidence of there being some difference in the paths by which the vaso-constrictor fibres reach the different abdominal vessels, since the local and the general effect do not run pari passu. Hence with these lower nerves, such as the 11th and 12th, the amount of the kidney contraction is out of proportion to the rise of general blood-pressure. Although the results obtained from these nerves are exceedingly constant, yet not unfrequently the "secondary shrinking" described above, accompanied by a fall of blood-pressure, is to be observed. Thus in one instance, Fig. 8, Plate XXIV., the excitation of the 12th nerve in the usual manner gave a large renal contraction and a moderate rise of blood-pressure. The latter soon returned to its normal level, and then fell markedly below this level, i.e. as much as 10 mm. Hg. This fall of general blood-pressure was accompanied, as seen in the trace, by a very considerable "shrinking" of the kidney. It will be noted that no diminution of the volume of the

kidney occurred with the fall of the blood-pressure to the normal height, although this fall was partly cardiac, as is seen by inspection of the trace, hence the kidney vessels must have been well contracted. On the other hand, as soon as the secondary fall of blood-pressure occurred, the kidney began to shrink immediately, so we must assume that in this case the kidney simply diminished passively in volume owing to the fall of general blood-pressure. As has been said before, the full discussion of this question of "secondary shrinking" must be reserved until the close of this paper, when the reflex phenomena and the results of excitation of the splanchnic nerve have been considered.

13*th Dorsal Nerve.*

A considerable number of observations were made with this nerve, but none of the various occasional results described above for the different nerves were observed. That is to say, this nerve gave uniformly great contraction of the kidney and a marked rise of blood-pressure. The renal contraction is not only marked and persistent but with this nerve no "secondary shrinking" or indeed any other form of mixed effect has been observed. In all cases, with the usual rate of excitation, pure contraction only has been observed. This fact is of very considerable importance, since although this nerve gives the most constant results on excitation it can be proved below to contain vaso-dilator fibres for the kidney in abundance. So that there must be some other explanation for the mixed effects observed with the upper nerves, besides the one assuming the existence of dilator fibres, and this explanation, as hinted above, is no doubt the fact that these upper nerves are distributed largely to other vessels than the kidney vessels.

With the 13th dorsal nerve we practically come to the last nerve producing any considerable effects, either on the general blood-pressure or on the kidney vessels.

1*st*, 2*nd*, *and* 3*rd Lumbar Nerves.*

As just mentioned the effects of the excitation of these lumbar nerves are but small, and frequently the 1st lumbar gives no effect on stimulation as regards the kidney vessels, but there is generally a slight rise of general blood-pressure. Thus in one case the 11th and 12th dorsal produced large effects, both on the kidney and on the general blood-pressure, yet the first lumbar gave no result on the kidney, and

but a small rise of general blood-pressure. The rise of blood-pressure with the 12th nerve amounted to 35 mm. Hg., that with the 1st lumbar to 10 mm. Hg.

In a few cases the 1st lumbar has given a slight renal contraction, but there are considerable variations in this respect. Hence we may conclude that although the 1st lumbar contains some vaso-motor fibres, yet in the majority of cases it does not send many to the kidney vessels. The same is true of the 2nd lumbar, the effects of the excitation of this nerve both on the kidney and on the general blood-pressure are as a rule very slight. In one case, however, this nerve produced a fair effect, but this is quite exceptional.

In no case has the excitation of either the 3rd or 4th lumbar nerve produced any effect on the volume of the kidney, and practically no effect (certainly no marked rise) has been observed on the general blood-pressure[1].

We may then conclude that the 2nd lumbar nerve marks the lower limit of the series of nerves, containing vaso-motor nerves for the kidney and other abdominal vessels. Just as, however, we found that there were variations in the position of the upper limit, so the same is true for the lower limit. In other words, the exact level at which the vascular fibres begin to leave, and cease to leave, the cord varies slightly in different cases.

Hence the most extended series of nerves supplying the renal vessels begins at the level of the 6th dorsal, and terminates at the level of the 2nd lumbar. However, it is only from the 10th to the 13th dorsal that the roots contain renal vaso-constrictor fibres in any great abundance.

Within the limits of this smaller zone the results of stimulation are practically constant; above and below this zone various abnormal results, so to say, are frequently observed. This has been fully described above.

II. Origin and Course of the Renal Vaso-dilators.
(Figs. 9, 10 and 11.)

Having thus described the origin and course of the vaso-constrictor fibres in some detail, and having mentioned the occasional abnormali-

[1] The results of excitation of these and the lower lumbar nerves will be described in a subsequent paper.

ties observed, we will now pass on to the question of the existence of renal vaso-dilators, before proceeding to discuss fully the significance of the most important variations already described.

Hitherto no definite evidence has been adduced in support of the view of the existence of vaso-dilators for the kidney vessels, although many observers have searched for them. With very few exceptions, e.g. chorda, nervi erigentes, it has been found much more difficult to demonstrate vaso-dilators than vaso-constrictors. A good example of this is of course the sciatic, with reference to which there have been many controversies, although now most observers would admit the existence in this nerve of both sets of fibres. In the case of the kidney vessels it is clear that it is much more difficult to demonstrate the existence of vaso-dilator fibres than of vaso-constrictor fibres. There are many more experimental errors to eliminate in the case of the dilator fibres. Thus in the case of the vaso-constrictor fibres all that is necessary is that a given stimulus should be followed by a renal contraction, accompanied by a rise of blood-pressure, or if the contraction is slight by no alteration of blood-pressure. In the case of the constrictor fibres it is scarcely necessary in many cases to observe the general blood-pressure, with the dilator fibres it is different however. In the first place, all observations must be controlled by synchronous observations on the general blood-pressure. Secondly, care must be taken not to confound a mere passive congestion of the kidney (due to contraction in the other areas) with an active and true expansion. In other words, in order to prove the existence of renal vaso-dilator fibres we must be able to produce at will a dilatation of the kidney unaccompanied by any rise of blood-pressure. That is to say, the renal expansion must be accompanied either by a fall of general blood-pressure, or else the general blood-pressure must remain unaffected during the local dilatation. It is clear that by this means the error of mistaking a passive congestion for an active dilatation is reduced to a minimum. Further, the renal expansion ought to be marked in amount, as if small it might be passive in origin and yet the mercurial manometer show no alteration of general blood-pressure.

It was for this reason amongst others that the oncograph was discarded, and a simple piston recorder used instead, as by this latter instrument no magnification of the kidney movements takes place[1].

[1] The apparatus consists of a glass tube fitted with an ebonite piston, a writer is attached at right angles to the piston rod, so that the movements of the piston, and hence of the oil, are recorded.

For this reason also I do not adduce as evidence of vaso-dilator fibres the various occasional and slight expansions of the kidney, unaccompanied by any obvious changes of blood-pressure, that have been described above as sometimes occurring on stimulation of some of the lower dorsal nerves. It is clear that before admitting the existence of vaso-dilator fibres for the kidney we must be able to produce active expansion of the kidney at will, and not rely simply on occasional experimental facts, since basing conclusions on occasional abnormal results is really a form of reasoning in a circle. Similarly, the statistical method is to be eschewed, e.g. because a certain proportion of cases gives abnormal results, therefore there must be two sets of nerve fibres.

At the present time there are practically three methods used in order to demonstrate the existence of vaso-dilator fibres in nerves which contain vaso-constrictor fibres as well, e.g. the sciatic.

Firstly, there is what may be called the anatomical method. Thus in the case of the sciatic, excitation of the roots of the lumbar plexus produces dilatation, whereas the stimulation of the abdominal sympathetic causes contraction of the same vessels. Hence, in this case, the constrictor fibres leave the spinal cord at a different and higher level than the dilator fibres for the same area.

Secondly, there is the degeneration or Wallerian method, i.e. the division of a nerve and the excitation of its peripheral end, some variable but short time after its section. By this method it is often possible to get evidence of dilator fibres after the degeneration of the constrictor fibres and before total degeneration of the nerve[1].

Thirdly, by different methods of excitation, i.e. the employment of slow rhythmical stimulation instead of the usual rapid interruption of the ordinary Du Bois Reymond coil.

In the case of the kidney, it is clear that the second of the above methods is not so practicable as either the first or third, although it is probably by no means impossible. For this reason the other two methods were used, and as yet no observations on the results of the excitation of nerves after previous section have been made. I trust, however, to make them shortly. Again, the third or stimulation method is probably the best in the case of the kidney, since the excitation of the nerves from the 6th dorsal to the 2nd lumbar by the ordinary method did not yield any evidence that could be adduced with certainty

[1] Bowditch, this *Journal*, Vol. VII.

in favour of the existence of vaso-dilator fibres. Nor did the excitation of this extended series of nerves give any clue to the dilators, if present, running in different channels to the constrictors, e.g. the posterior roots for instance. The occasional dilatations observed were not more frequently seen with any one nerve, although on the whole they were most frequently absent with the lowest nerves. As mentioned before, these results could not be considered as in any way giving good evidence of the existence of vaso-dilators. Hence the anatomical method did not seem a promising one. There remained the stimulation by slow rhythmical shocks, and this method fortunately gave clear and unmistakeable proof of the existence of these dilator fibres. As previously mentioned, for exciting purposes when slow rates were employed, either a vibrating reed with Helmholtz's modification was used, giving rates varying from 50 to 5 per second, or if a slower rate was needed, an apparatus interrupting the primary current once per second was introduced into the circuit, and by a contrivance only the break shocks were used, the make shocks being short circuited. Before considering the effects of such slow excitation as 1 per second it will be necessary to describe briefly the results when the rate of excitation varies from 50 to 5 per second; for the purpose of avoiding unnecessary repetition we will describe the effects when such a nerve as the 12th or 13th is the one stimulated. Any differences between these nerves and the others will be considered subsequently. These two nerves are chosen because, as mentioned above, they contain more fibres distributed to the renal vessels than any other nerves. No difference is to be detected in the results when the rate of excitation is slowed from 50 per second to 20, or even 10 per second. Even with this comparatively slow rate the blood-pressure rises considerably, and the renal vessels contract. Although as mentioned in a preceding part of this paper there are occasional differences between the length of the latent period of the renal contraction and that of the rise of blood-pressure, yet in the majority of cases, and in what may be called the typical case, the duration of the latent period of the two effects is sensibly equal, and it is roughly about 2″. Frequently, however, it is shorter than this, and but rarely longer unless the animal is exhausted by hæmorrhage shock, prolonged stimulation, &c. With slow rates of stimulation, however, marked effects are produced on the latent period, as is seen by the following experiment. In this case the 13th dorsal nerve, prepared in the usual manner, was excited with a given strength of current interrupted 50 times per second. The stimulus was then repeated, but

with a slower rate, i.e. 20 per second instead of 50. In the two cases practically equal results were obtained, both on the kidney and on the general blood-pressure. The latent periods of the two effects were practically the same, and were equal to 2″. The renal contraction was marked and persistent, and there was neither a secondary fall of blood-pressure nor any "secondary shrinking" of the kidney after the cessation of the stimulus. On repeating the excitation with the same strength of current, but with the rate diminished to 5 per second, very different effects were observed. A very slight contraction of the kidney occurred, and not only was this contraction very transitory in its duration, but its latent period was some 4″. The rise of blood-pressure still took place, although to a diminished extent, but its latent period was still 2″. Hence there was a difference of some 2″ in the latent periods of the two effects. In another case not only was there a difference between the latent periods of the two effects amounting to 2″ but the latent period of the renal effect was 11″, that of the blood-pressure rise being 9″. Previously with a quicker rate of excitation the duration of the latent periods had been 2·5″. In both these instances the rise of general blood-pressure was out of proportion to the renal contraction, which was of a slight and transitory character. In another instance with a rate of excitation of 10 per second the latent period of the renal contraction was 2·5″; with a rate of 2 per second this was increased to 5·5″. In the former case, with the quicker rate, the rise of blood-pressure was considerable, and the renal contraction marked and persistent. With the slower rate, the rise of blood-pressure was much less, and the renal contraction very small in amount, and instead of being persistent it gave way to a fairly well marked dilatation. In other cases the slowing of the rate of stimulation has prolonged the latent period to as much as 8″, or even more. From the consideration then of the above results it will be seen that when the rate of excitation falls to about 5 per second, not only are the effects less marked in amount, but the renal contraction is more altered than the rise of blood-pressure. Nothing is more remarkable than the fact, that although with the slower rates the renal contraction is not only less in amount but also so much more transitory, yet notwithstanding this there is frequently a considerable rise of blood-pressure, although of course not so much as with the quicker rate of stimulation. Further, and this is equally important, the latent periods of the two effects are no longer of equal duration. Lastly, the effect on the kidney is frequently a mixed one, the contraction being followed by expansion. This last result

is one which practically scarcely ever occurs with ordinary methods of excitation, whereas it is quite common with these slow rates.

As yet, however interesting the above results may be, we have not adduced any very positive evidence in favour of the existence of vaso-dilator fibres. This, however, is at once obtained when the rate of stimulation is still further diminished, so that break shocks only are sent into the nerve at a rate of 1 per second or even 1 in two seconds.

When such a nerve as the 13th dorsal (which with quick rates of stimulation always gives a renal contraction and a rise of general blood-pressure) is excited with these slow rates, the kidney undergoes marked expansion, and there is no rise of blood-pressure. The blood-pressure remains either at its previous height or else undergoes a slight fall. Hence by this difference in the mode of excitation the same nerve can be made to give exactly opposite results. This is not an occasional phenomenon but it is perfectly constant. Just as the quick rate of excitation invariably produces contraction of the kidney, so does the slow rate cause an expansion. This expansion of the kidney is well marked, as will be seen by reference to Figs. 9 and 10, Plate XXIV. It must be remembered that a piston recorder was used in this experiment, so that there was no amplification of the kidney movement, such as is seen with the oncograph. It is true that the expansion is not so great in amount as the contraction obtained by the stimulation of the same nerve with a quick rate. This, however, can scarcely be expected, since in all probability the slow rate excites not only the dilator fibres, but also the constrictor fibres to some slight extent, so that the resulting dilatation is simply a preponderance of the one effect over the other. It may be said that this argument cuts both ways, but quick rates apparently do not excite dilator fibres in mixed nerves, or at any rate do not excite them to any appreciable extent. Further, the quick rate no doubt is a better stimulus to the constrictor fibres than the slow rate to the dilators. At any rate in all mixed nerves, e.g. the sciatic, it is much more difficult to obtain marked dilatation than marked constriction, and no doubt the same is true for all nerves containing mixed vaso-constrictor and vaso-dilator fibres. Although the expansion is not so great in amount as the contraction, yet it is of considerable amplitude, so that no doubt can be entertained of its active nature, when it is remembered that there is no synchronous change of blood-pressure capable of causing it passively. This renal expansion has a short latent period, and the rate of expansion is slow, considerably slower than the rate of contraction for the same nerve. Like the contraction effect,

however, the expansion is of a persistent character, and generally the kidney remains for a variable time more expanded than it was previously to the stimulation. There is thus an after-effect in the same direction as the excitatory change, both when the kidney contracts and also when it expands; in the former case it remains more or less permanently partially contracted, in the latter case partially expanded. We may, then, sum up the facts with regard to such a nerve as the 13th dorsal in the following way. Quick rates produce contraction, slow rates expansion, of the kidney. With the former effect the general blood-pressure rises, with the latter effect it remains either constant or else a slight fall occurs. With intermediate rates, e.g. 5 per second, a slight rise of blood-pressure is produced, and a very small contraction of the kidney of transitory duration.

From these results, then, we may conclude that the 13th dorsal contains not only vaso-constrictor fibres for the vessels of the kidney, but also that vaso-dilator fibres are present in abundance.

The same results are seen with the 12th nerve, and the expansion produced by slow rates is quite as marked with this nerve as with the 13th, so that this nerve also contains dilator fibres for the renal vessels.

When we pass to the higher nerves somewhat different results are obtained with slow rates, results essentially the same as those seen on excitation of the splanchnic, with the same slow rates. In the first place it is rare to obtain a renal dilatation with these nerves, it is more common to get either the familiar rise of blood-pressure with a mixed renal effect, or else for no effect to be apparently produced by the stimulus, and then after a long latent period to obtain a slight fall of blood-pressure, accompanied by a passive shrinking of the kidney. It will be more convenient to consider these results when we are discussing the results of the same mode of excitation on the splanchnic nerve. It is however from the 10th nerve upwards that the results are similar to those obtained from the splanchnic. The 11th nerve gives results like the 12th and 13th, but not quite so marked.

No effects have as yet been obtained by the slow stimulation of the lumbar nerves, although it is probable that sometimes results might be obtained from the 1st lumbar, similar to those obtained from the 13th dorsal.

At first sight it may seem somewhat unsatisfactory that a true active dilatation of the kidney is only to be obtained by the stimulation of such a comparatively restricted series of nerves as the 11th, 12th and 13th dorsal. A moment's reflection will show, however, that these are

the very nerves that have been shown above to be those containing most of the vaso-constrictor fibres that are distributed to the renal vessels. Hence finding the most definite evidence of vaso-dilators where the vaso-constrictor fibres are the most abundant, is very clear evidence that the two sets of nerve fibres do not pursue different paths to reach the kidney vessels. No doubt vaso-dilator fibres run also in the 1st lumbar occasionally, just as it has been shown above that vaso-constrictor fibres do, but, as was mentioned, there are considerable variations in different cases, in regard to this nerve. Frequently no vaso-constrictor fibres can be demonstrated in it, and this is usually true for dilator fibres also. No doubt the excitation of the 1st lumbar by slow rates in a large number of cases would occasionally produce dilatation, but as yet in the comparatively small number of observations made on this nerve no dilatation was obtained. Inasmuch as such definite evidence was obtained with the 11th, 12th, and 13th nerves where the constrictors are so abundant, it was not thought worth while to make a long series of experiments on the 1st lumbar, where constrictor fibres are so scanty and variable.

Having thus determined the existence of vaso-dilator fibres for the renal nerves in those spinal roots where vaso-constrictor fibres are most abundant, there remains the question as to why the middle dorsal nerves, i.e. from the 6th to the 10th, do not produce renal expansion with slow rates of excitation, seeing that quick rates of excitation produce renal contraction. This question will be most easily settled by the consideration of the phenomena following excitation of the splanchnic nerve.

III. The Phenomena following Excitation of the Splanchnic Nerve. (Figs. 12 and 13.)

This nerve was exposed from the back, by making an incision an inch and a half long at the outer border of the erector spinæ, over the last two ribs and on the side desired. The sheath of the muscle is opened and the muscle itself separated from its attachments in the limited area exposed and removed *en masse*. All bleeding vessels are seized with Spencer Wells' forceps and twisted or tied. The 11th or 12th rib was then resected, or at least an inch of it removed, and then the splanchnic nerve can be easily reached, ligatured, divided and the peripheral end arranged for excitation. As a rule the nerve was stimulated above the level of the 11th rib, that is to say, above the

ramus communicans from the 11th spinal nerve. Hence the nerve at the point of excitation contained the fibres that it had received from the spinal nerves, down to and including those derived from the 10th dorsal.

When a stimulus of moderate intensity and quickly interrupted is applied to this nerve, the familiar facts of a rise of general blood-pressure accompanied by a marked renal contraction are observed. This, of course, was observed long ago, and carefully investigated by Roy[1] amongst others. The rise of blood-pressure is of course large, and as a rule it is more persistent than when a single nerve root is excited. On the other hand, the total rise of pressure may not be greater than when a single root is strongly excited, of course as a rule it is greater with the splanchnic than with a single root. The contraction of the kidney is also of course very marked and long-continued, but occasionally this contraction is followed by a slight expansion. This expansion, however, occurs only some minutes, e.g. two after the cessation of the excitation. In other words, the organ remains with its vessels slightly less contracted than previously to the excitation. It in no way resembles the active dilatation described above, as occurring when certain nerve roots are excited by means of slow rhythmical shocks. It differs from these effects by the great length of time between the excitation and its occurrence. In one, the splanchnic was stimulated for five seconds, and this after-effect of permanent expansion occurred between two to three minutes after the cessation of the excitation. This after-effect of renal dilatation is probably not due to the stimulation of vaso-dilator fibres, since there is no fall of general blood-pressure, although this would not occur unless the dilatation was not only extensive but also considerable in amount. We will return to this point later on, at present it is sufficient to note the fact that the renal contraction is sometimes followed by an after-effect of dilatation. These facts are of course not new, and they have only been inserted here, partly for the sake of completing the account, and partly as a contrast to the effects following slow excitation of the same nerve with the same strength of current.

When the splanchnic prepared as above is excited by slow rhythmical shocks, i.e. 1 per second instead of 50 per second, a fall of general blood-pressure is observed instead of the rise that is so well known to follow ordinary excitation. This fall of general blood-pressure is well marked, amounting in many cases to 15 to 20 mm. Hg. Although well

[1] Roy, *Virchow's Archiv*, 1883.

marked it is slow and gradual in its course, resembling in this respect a depressor curve, and contrasting markedly with the sudden rise produced by ordinary rapid stimulation. This fall of blood-pressure persists during the excitation, but on shutting off the current a sudden rise of pressure is frequently seen to occur. This rise, however, is but slightly marked, that is to say, the blood-pressure curve does not rise much higher than its previous normal height. Further, this rise is of but transitory duration, and hence contrasts with the fall. As a rule this sudden rise of the pressure lasts but some 10″, and then the pressure falls to its previous low level, that is to say, sinks below the normal, and then slowly regains the height at which it stood previously to the excitation. This sudden elevation of pressure, occurring so to say on the top of the fall of pressure, is not always to be observed, and apparently it is not entirely dependent on the amount of the preceding fall. Thus in some cases a slight fall is followed by a considerable after-rise, in others a considerable fall by a small after-rise. In all cases, however, slow rhythmical excitation produces a fall of general blood-pressure, although, as just mentioned, the return of the pressure to the normal height is frequently not interrupted by any sudden rise. The fall of pressure may in some cases be very slight, e.g. 5 mm. Hg., but frequently it may amount to 20 mm. Hg. Owing to the mode of preparation this result is clearly due to a peripheral effect, and not to any reflex excitation, since by exposing the nerve from the back a long length of it is obtained, and there is no danger of any spreading of the current to the central end. Hence we must assume that the results are due to the excitation of certain efferent fibres in this nerve.

The examination of the behaviour of the kidney, whilst the above changes are seen in the blood-pressure, throws considerable light on their significance. During the fall of aortic pressure the kidney shrinks slowly and gradually, and during the subsequent sudden and transitory rise the kidney undergoes an equally sudden and transitory expansion. Thus the kidney curve is exactly similar to the blood-pressure curve, and so we must conclude that, inasmuch as the changes in the volume of the kidney are such as would be produced by the blood-pressure alterations, the renal vessels are only affected indirectly and in a passive manner. It is obvious that if the shrinking of the kidney were due to an active constriction of its vessels the blood-pressure ought to be raised. On the other hand, if the nerve excitation has caused a peripheral dilatation it might be expected that the kidney would expand. This, however, is only true, if in the first place the kidney vessels are concerned in the

dilatation, and secondly if there is no great dilatation elsewhere. If, for instance, all the abdominal vessels dilate, it is quite possible for the kidney to diminish in bulk, even if its own vessels took part in that dilatation, owing to the large quantity of blood diverted into the other abdominal vessels, e.g. the intestinal vessels. The question as to whether the kidney will expand or not, is one that is decided not only by the changes occurring in the renal vessels, but the ratio of this change to that occurring simultaneously elsewhere.

Similarly it is clear that the sudden expansion of the kidney during the transitory rise of pressure after the cessation of the excitation is not the cause but the effect of that rise of pressure. Fig. 13, Plate XXV. Hence we can explain the fall of pressure and the shrinking of the kidney as both being due to a peripheral dilatation caused by the rhythmical excitation, and this dilatation is mostly produced in all probability in the intestinal vessels. Although the renal vessels are affected passively, it is probable that really some active dilatation occurs in them also, but its effects are quite masked, owing to the small size of the renal area compared to that formed by the rest of the abdominal vessels.

We must conclude then that the splanchnic nerve contains not only vaso-constrictor fibres but also vaso-dilator fibres. Although the above experiment clearly demonstrates that this nerve contained vaso-dilator fibres for the abdominal vessels, yet it might be thought that it gave no evidence of renal vaso-dilators in the splanchnic. In no case has excitation of this nerve caused an expansion of the kidney unaccompanied by a rise of blood-pressure. On the other hand, if the curves obtained with slow excitation are examined, it is seen that although the kidney is shrinking, yet the heart-beats are much better marked on the kidney trace during this shrinking than they are on the trace previously to the excitation. This again affords evidence, if it were needed, that this shrinking is not due to an active contraction of the renal vessels, but that it is a passive effect due to dilatation produced elsewhere. At the same time this increased visibility of the heart-beats tends to show that the renal vessels themselves were less rigid, i.e. that they also had become dilated as a result of the excitation, but that for the reasons given above, this renal dilatation was not associated with an actual increase in the volume of the kidney.

It is of course to be understood that in all the above instances rapid excitation of the nerve caused as usual renal contraction and heightened arterial tension.

Having thus demonstrated by means of slow rhythmical excitation that the splanchnic contains vaso-dilator fibres for the abdominal blood vessels, the renal vessels amongst others, we will now consider the effects of excitation of the splanchnic by intermediate rates, the above results being obtained only if rates as slow as 1 per second are used, or in many cases still slower rates are necessary, e.g. 1 in two seconds.

If the nerve is excited by stimuli a little more frequent than the above, but not more than two per second, it will be found that practically no effect is produced during the excitation, unless this be prolonged to 40″ or thereabouts. On shutting off the current, however, if the stimulation has not been very long, or after a long latent period if the nerve be excited for say 40″, a slight fall of blood-pressure occurs, accompanied by a passive shrinking of the kidney. That is to say, essentially the same results are obtained, but their latent period is longer and their amount is less. It is important to note that these effects are also produced on shutting off the current, when during the excitation no effect has been produced, either on the kidney or the blood-pressure. In these cases the phenomena are practically the same, as what has been described above as the "secondary shrinking" of the kidney, seen when certain nerve roots were excited in the usual manner with quick rates. With very slow rates then these phenomena occur during the stimulation, with slow rates they occur when the current is shut off; with quick rates they are only occasionally seen, and then only after cessation of the excitation. In all three cases the phenomena are essentially similar, i.e. a fall of blood-pressure due to peripheral dilatation. There is, however, this important point, that whereas with quick rates these effects are preceded by those dependent on peripheral contraction, with slow rates they occur without any such preceding constriction. Dastre and Morat[1] have observed the same phenomena in other cases. They find that the excitation of a so-called vaso-constrictor nerve produces of course constriction of the vessels to which it is distributed, and that afterwards this is followed by dilatation. To this dilatation they gave the name of *surdilatation*, but it is clear that it is really the same condition that I have called in the kidney "secondary shrinking," in order to point out its difference from an active contraction, although in both cases the volume of the kidney diminishes. Hence this "secondary shrinking" of quick excitation being due to peripheral dilatation in a large area, and being the same as the *surdilatation* of the French observers, there remains the question

[1] Dastre et Morat, *Système Nerveux Vaso-Moteur*, p. 23.

as to its nature. The French observers consider it is an exhaustion phenomenon, since they only observed it after preceding constriction. They considered that the excitation increased the tonic contraction of the muscular wall of the vessel, and that then the muscle became exhausted, and hence ensued a temporary dilatation. This explanation is no doubt a simple one, and one quite capable of explaining the results when these are the after-effects of a preceding contraction, such as is the case when quick rates of excitation are used. It has been shown above, however, that with slow rates the same effects are seen without any preceding contraction, and with very slow rates these effects are increased in amount, and are really the results of the excitation of dilator fibres. It is clear that with slow rates they cannot be exhaustion phenomena, since no active process has preceded them. Hence we must conclude that in all cases they are the result of the stimulation of dilator fibres. We may then further conclude, that where these effects are seen after ordinary excitation of a so-called vaso-constrictor nerve we are in reality exciting a mixed nerve, containing both vaso-constrictor and vaso-dilator fibres.

When such a mixed nerve is excited with either very slow or with quick rates, although opposite effects are produced, yet in all probability both sets of fibres are really excited, only in one case one effect preponderates, in the other case the other. Not only is this the case, but sometimes there is a tendency for the one effect to be produced during the passage of the current, and the second or opposite effect to occur when the current is shut off. Thus we have seen that with quick rates the dilator effect occurs after the cessation of the stimulus. With slow rates the same is sometimes seen, i.e. when the current is shut off a slight constrictor effect. An example of this is the transitory rise of pressure described above, as occurring sometimes on slow excitation of the splanchnic. These opposite effects of slow and quick rates of excitation, and also the results seen on shutting off the current, are phenomena by no means confined to mixed vascular nerves. In the case of gland nerves[1] I have shown that slow and quick rates produce opposite electrical variations, and also that sometimes no effect is produced during the passage of the exciting current, but that an effect both electrical and secretory is seen when the current is shut off. What the explanation is we do not know, but although in mixed vascular nerves slow rates of excitation cause dilatation, and quick rates

[1] Bradford, this *Journal*, Vol. IX., p. 290.

contraction, it is probably not true that the latter only excites vaso-constrictor fibres, and the former only vaso-dilator fibres.

Finally, we conclude that the splanchnic nerve contains not only vaso-constrictor but also vaso-dilator fibres for the vessels of the abdominal viscera and for the renal vessels amongst the rest. There are, however, more dilator fibres for the renal vessels in the roots of the lowest dorsal nerves, as shown above. We must further conclude that there is no evidence to show that the renal vaso-dilators reach the kidney vessels by different paths to those followed by the constrictors, and just as the renal constrictors are most abundant in the lowest dorsal roots, so are the dilators.

IV. The Phenomena following Excitation of the peripheral end of the Divided Vagus.

Stimulation of this nerve in the neck causes of course marked contraction, or rather shrinking of the kidney, owing to the action of this nerve on the heart. It is well to remember that the inhibitory action of the vagus is not abolished by the doses of curare used in this research, which were only sufficient to paralyse the motor end plates. After the injection of a small dose of atropin excitation of the cervical vagus causes no effect on the volume of the kidney. Excitation of the nerve in the thorax after it has given off its cardiac fibres also produces no effect on the volume of the kidney. Hence the vagus does not supply any fibres to the vessels of the kidney. These results are of course quite in accordance with the facts observed by Roy[1], and are only cited here in confirmation of his results, since recently some French observers[2] have obtained diminution in the flow of urine as a result of vagal stimulation. However, inasmuch as this nerve, at any rate in the dog, gives no vascular fibres to the renal vessels, the discussion of this result must be postponed to a future paper, where I hope to consider the phenomena of the renal secretion, here we are only concerned with the renal circulation, and the vagus has no action on this apart from its cardiac action.

[1] Roy, *Virchow's Archiv*, 1883.
[2] Masius, *Centralblatt für Physiologie*, No. 23, 1889.

V. The Reflex Phenomena of the Renal Vessels. (Figs. 14 to 20.)

We have to consider under this heading not only the sciatic, vagus, depressor and intercostal nerves, but also the effects following the stimulation of the central ends of the posterior roots of the nerves, which have been shown above to supply the kidney. We will, however, begin with the sciatic.

(*A.*) *Results following excitation of the central end of the sciatic.*

When the central end of the divided sciatic is stimulated, the excitation is followed, as has been shown by Roy, by a well-marked contraction of the kidney, accompanied by a marked rise of general blood-pressure. I have very little to add to this, which is the usual effect. The kidney contraction is a sudden one, it is also persistent and the blood-pressure rise is of course marked, and there is no after-fall of pressure in by far the greater number of cases. Very rarely, in fact only once or twice, the rise of blood-pressure has been accompanied by a slight expansion of the kidney. This result was observed on a rabbit, but in this case neither the effect on the kidney nor that on the blood-pressure was at all marked, and it is more than probable that the slight renal expansion was not active, but was a passive effect due to simultaneous rise of pressure. So that contraction of the kidney following this excitation is a most constant result, as in no case has any marked expansion been observed.

In some cases of course excitation of this nerve is followed not by a rise of blood-pressure but by a marked fall, amounting in many cases to as much as 25 mm. Hg. This effect was perhaps most marked in some experiments on this nerve in a cat. In this case the stimulation of the sciatic was followed by a fall of as much as 26 mm. Hg. Along with this fall of blood-pressure the kidney underwent a considerable contraction, as is seen in Fig. 20, Plate XXVI. We may conclude that the reflex dilatation causing the fall of blood-pressure occurred in a large area, and that the kidney vessels were only secondarily affected. In some cases, however, the character of the kidney contraction seemed to show that its own contraction was an active one, and that the fall of pressure was hence due to a large dilatation elsewhere. Other facts pointing to the conclusion that the excitation of this nerve affects other areas more

markedly than it affects the kidney vessels, were obtained in this same animal. Thus sometimes this nerve caused a rise of blood-pressure accompanied by a renal contraction, and at other times a slight rise of blood-pressure followed by a fall was seen. In this last case during the rise of pressure the kidney volume was unaffected, but synchronously with the fall the kidney underwent a marked passive contraction. This result is most easily explicable on the hypothesis that the rise and fall of pressure were produced mainly by vascular changes, not occurring in the kidney vessels but probably produced in the large area of intestinal vessels.

It is clear from the consideration of the above and of many other experiments that the reflex phenomena are very complicated, and no doubt the results obtained depend largely on the condition of the centre at the moment when the stimulus from the periphery is received. It is certain, however, that practically in all cases the kidney undergoes contraction, generally active but sometimes passive. This point is one of importance and hence the stress laid on it here. Further it is probable that the kidney vessels are less affected by the reflex stimulation than many other vessels, and more especially the intestinal vessels.

(*B.*) *Results following excitation of the central end of an intercostal nerve.*

The excitation of the central end of a divided intercostal nerve is followed by a slight rise of the general blood-pressure, accompanied by a contraction of the kidney. The effects, however, are not large, the rise of blood-pressure being generally about 15 mm. Hg., and the contraction of the kidney is by no means considerable. In no case, however, has any other effect than contraction of the kidney been observed. In a few instances a fall of blood-pressure has been observed on the excitation of these nerves, but in these cases the kidney volume was not being registered; from analogy, however we may conclude that this fall of blood-pressure would be accompanied by a passive shrinking of the kidney.

(*C.*) *Results following excitation of the central end of the vagus.*

The results of the excitation of the central end of the divided vagus (the opposite vagus being cut) have been observed in the dog, cat and

rabbit. The stimulation is followed by a rise of blood-pressure, accompanied by a contraction of the kidney of the usual character. If, however, the excitation be frequently repeated it will be found that the effect on the kidney undergoes considerable alteration, at a time when the stimulus still causes a rise of blood-pressure, although this may be less marked in amount than previously. That is to say, that although the rise of blood-pressure is due to a peripheral constriction of vessels, the kidney vessels soon do not take part in this constriction, or if they still do so, it is to a markedly less extent than previously. Hence in some cases the change in the volume of the kidney is a mixed one, and occasionally the kidney undergoes a slight expansion during the heightening of the general blood-pressure. As a rule, however, the kidney undergoes a pure contraction provided the blood-pressure simply rises.

(*D.*) *Results following excitation of the central end of the depressor.*

The effects following the excitation of the central end of this nerve were observed in rabbits, but very frequently the stimulation of the central end of the divided vagus in the cat gives very good and typical depressor effects, so that the following statements apply quite as much to the cat as to the rabbit. In the dog it is but rarely that a depressor effect is seen to follow stimulation of the central end of the vagus, but in the cat this result is in my experience more common than the rise of general blood pressure.

Both in the cat and rabbit the first point that is striking is, that although the excitation causes a very great fall in the general blood-pressure, yet the effects on the kidney are comparatively very small, much less for instance than the effects produced by stimulation of the central end of the sciatic. In by far the greater number of cases the great fall in the general blood-pressure is accompanied by a comparatively small diminution in the volume of the kidney. The blood-pressure curve and the kidney curve follow one another exactly, so that it is clear that the kidney vessels do not undergo any very marked expansion or contraction. At the same time the diminution in the volume of the kidney is slight, when compared to the great fall of arterial tension. This is well seen by comparing Figs. 14 or 15, Plate XXV. with Fig. 20, Plate XXVI. Although the fall of pressure following sciatic excitation is less than that obtained with the depressor, yet the renal effect in the latter instance was less than in the former.

In some cases in the rabbit, notwithstanding that the depressor causes a great fall of arterial tension, the volume of the kidney remains unchanged, and in a few cases the kidney has even undergone a slight expansion. We must conclude that in both these instances the kidney vessels must have expanded somewhat, so as to neutralize the effects of the great fall of pressure on the kidney. It is clear that the kidney vessels cannot be altogether passive, because if they were the great fall of blood-pressure would cause a passive shrinking of the kidney. Hence we must conclude that when this nerve is excited the renal vessels really dilate somewhat, but that owing to great dilatation produced at the same time elsewhere this renal dilatation is unable to manifest itself by any increase in the volume of the kidney. Hence a passive shrinking of the kidney of variable amount is produced, owing to the accumulation of blood in the vessels of other areas, and probably more especially in the intestinal vessels. Thus the volume of the kidney is really but little affected, comparatively speaking, by the reflex effects produced on excitation of the depressor. This result is not to be taken as showing that the renal vessels are not actively affected by the excitation, but rather as showing that the active dilatation of the renal vessels is more or less neutralized by the dilatation occurring elsewhere. That this is so is shown, as we saw before, by the smallness of the passive shrinking. In some cases this passive shrinking of the kidney is somewhat greater, and this is readily explained by assuming that the dilatation of the intestinal vessels is relatively greater than that of the renal vessels, and hence the great passive shrinking of the kidney. Thus we must conclude that although some dilatation of the renal vessels occurs, the actual effect produced on the volume of the kidney depends entirely on the relation this renal dilatation bears to the dilatation elsewhere.

(*E.*) *Results following excitation of the central ends of the posterior roots.*

We now turn to the consideration of the reflex results following excitation of the central end of a divided posterior root. As might be expected the results of this reflex excitation are not by any means so constant as the effects following the stimulation of the peripheral ends of the same nerves. The severity of the operation, the exposure of the cord, the hæmorrhage, the shock and anæsthetic, all these must influence the delicate mechanism of the reflexes far more than they do the peripheral effects. Hence it is not surprising to find some considerable

variations in the phenomena. As regards the effects of this excitation on the general blood pressure there is not much variety in the results obtained. In by far the greater number of instances the excitation of the central end of a posterior root is followed by a great rise of general blood-pressure. The latent period is a short one, and the rise is one of great suddenness, the maximum rise being soon reached. In this respect the rise is a very different one to that observed on excitation of the peripheral end of the same nerve for instance. The gradual rise produced by the latter mode of excitation contrasts greatly with the extremely sudden character of the reflex rise. Sometimes the rise of blood-pressure is so sudden that no respiratory undulations are marked on the rising curve, the tracing being almost a vertical line. It is, however, on the whole exceptional for the rise to be so sudden as this. This great and rapid rise only lasts for a few seconds, but generally the blood-pressure does not return completely to its previous level. That is to say, it remains at a slightly higher level to that it occupied before the excitation. This after-effect is of a permanent nature, so that if the blood-pressure is low at the beginning of an experiment it is quite possible to get it materially and permanently raised by a few excitations of the central ends of posterior roots, or by applying the electrodes to the posterior surface of the cord. Hence the reflex excitation causes not only a sudden and great contraction of the vessels, but also braces them up more or less permanently by the production of a more or less permanent tonic contraction. There is no very material difference to be observed, with regard to the effects produced on the general blood-pressure, whether the upper or the lower dorsal nerves are the ones whose posterior roots are excited. A rise of pressure is observed on excitation with all the nerves, and although the rise may be greater when the lower nerves are excited, yet there is not the marked difference between the upper and the lower nerves that was described above as occurring on excitation of the peripheral ends of these nerves. In some very exceptional cases, as will be mentioned more fully below, a fall of blood-pressure is observed instead of a rise, but this is very uncommon, and in fact during the course of a large series of observations this fall has only been observed in one animal.

When we turn to consider the effect of these excitations on the kidney we find that there is some want of uniformity in the results. In a large proportion of cases excitation of the central end of a divided posterior root is followed by great expansion of the kidney. This, although the common result, is by no means the uniform result. Thus

in some cases the kidney undergoes great contraction, and in others the results are mixed, first a contraction, then a dilatation. Even however where a marked contraction is produced this is usually of quite temporary duration, and it is quickly followed by expansion after the cessation of the stimulus. In other instances the expansion occurs after a very brief contraction, and therefore long before the cessation of the excitation, so that the kidney result is truly a mixed one. The expansion of the kidney is considerable in amount, but is usually not of a very persistent nature. Not only is there some variability in its occurrence in different animals, being present in the one and not in the other, but there are some differences with different nerves in one and the same animal. Thus in one case, although excitation of the central ends of both the 12th and 13th dorsal nerves gave marked rises of general blood-pressure, the former produced expansion of the kidney, the latter an equal contraction. In both cases the effects were very marked. Similarly it is not at all uncommon for the lowermost nerves, such as the 3rd and 4th lumbar, to cause contraction of the kidney, whereas the middle series, from the 8th to the 13th, cause expansion. This expansion, however, with the uppermost of these nerves is generally preceded by a slight contraction. Again, the uppermost dorsal nerves most commonly produce contraction. Hence we see that there are considerable variations in the results obtained with different nerves in the same animal. Finally, the same nerve can give not only in different animals different results, now expansion, now contraction, but these differences can also be obtained in the same animal and with the same stimulus. No doubt, as mentioned before, these differences depend quite as much on the condition of the vasomotor centre in the medulla at the moment of excitation as on any differences of fibres, &c. in the particular nerve excited. But the fundamental fact remains, that this is the only form of reflex excitation that causes expansion of the kidney, the sciatic, intercostal and vagus cause contraction, and the depressor, although causing expansion, has its renal effects neutralised and only a passive contraction of the kidney is produced. It is obvious that the expansion occurring on excitation of the central end of a posterior root is a local effect, and that there is a general effect in the opposite direction, i.e. constriction. This must be so to account for the rise of general blood-pressure that accompanies this renal expansion.

When expansion of the kidney and rise of blood-pressure are produced by this mode of excitation, it is found that there is always very copious hæmorrhage from the wound, necessary for the exposure of

the spinal cord. Now this hæmorrhage is frequently so profuse that the large wound fills almost at once with blood, although previously to the excitation it may have been perfectly dry. No doubt this hæmorrhage is largely due to the rise of blood-pressure, but that this is not its sole cause is seen from the fact that equal rises of pressure produced in other ways do not cause anything like such copious bleeding. Thus in many cases the central end of the sciatic will cause a rise of blood-pressure quite equal in amount to that obtained by excitation of the central end of a posterior root, but at the most there is only some oozing from the wounded vessels, never that sudden filling of the cavity with blood. I think we must conclude that although the rise in general blood-pressure does contribute to some extent to its causation, this hæmorrhage is largely due to an active dilatation of the vessels of the abdominal wall, similar to that occurring in the kidney. In other words, the stimulation of the afferent fibres in the given posterior root causes a local vascular dilatation not confined to the viscus from which it is conveying afferent fibres, but also in the area of the body wall supplied by it. No doubt this view may meet with some opposition, but it is difficult to believe that this copious hæmorrhage is due to anything but a local dilatation plus a general rise. Further, it is not observed unless the kidney undergoes dilatation, i.e. it does not occur with the same nerve and the same stimulus if a renal contraction is produced instead of a renal expansion.

Not only then does the central end of a posterior root tend to produce a local dilatation of the kidney, but, as hinted above, this effect is most often seen with the lower dorsal nerves, i.e. with those nerves that have previously been shown to contain most of the efferent fibres distributed to the kidney vessels. Since, however, all the dorsal nerves from the 2nd downwards contain some fibres distributed to the kidney, this reflex dilatation may be seen in excitation of the central end of any posterior root in this series. It must be noted, however, that this expansion is not seen with the posterior roots of the lumbar nerves, even when the dorsal nerves of the same animal yield it on excitation.

All these points show that the effect is a local one; and in this respect the renal dilatation resembles the dilatation seen in the case of the ear, the skin of the leg and the vessels of the stomach, when the posterior auricular, the saphenous and the central end of the vagus respectively is excited.

In connection with the local nature of the dilatation it is to be remembered that the central end of an intercostal nerve causes on

excitation a contraction of the kidney, whereas the central end of the posterior produces the dilatation. In both cases there is a rise of blood-pressure, although in the former case it is less in amount than in the latter case. Hence we must conclude that the expansion is due to the excitation of the afferent fibres coming from the viscera, as clearly the anatomical difference between the central end of an intercostal, and the central end of a posterior root, is that the latter contains not only the afferent fibres of the former, but also those of the viscera in addition. In one case, as previously noted, the excitation of the central end of a posterior root caused a great fall of blood-pressure, accompanied by a passive shrinking of the volume of the kidney, as is well seen in Fig. 19, Plate XXVI. The fall of general blood-pressure was in this case very great, and hence the shrinking of the kidney was also considerable. It will be seen by reference to Fig. 19 that the latent period of this effect is a long one, and in some cases this latent period was so long that the depressor effect was preceded by one of an opposite kind. That is to say, a rise of blood-pressure accompanied by an active renal contraction, in other instances, however, this depressor effect was not preceded by any such active effect. In these respects these reflex depressor effects resemble the dilator effects produced by slow excitation of the peripheral end of the divided splanchnic or of some of the lower dorsal nerves. Another point of resemblance is, that in both cases the effects are sometimes seen to follow the shutting off of the exciting current.

Depressor effects, as mentioned above, are also sometimes seen on excitation of the central end of an intercostal nerve.

These depressor effects obtained with either the central end of an intercostal nerve or of a posterior root are of interest, as serving to show how greatly the general blood-pressure can be lowered reflexly by the excitation of only one nerve. What the conditions are which lead to the stimulus causing such a profound depressor effect instead of the usual one are unknown; no doubt it is some central difference. This is confirmed by the great length of the latent period in many cases. Still the important fact remains, that occasionally these depressor effects are observed, and that they are, or may be, greater in amount than when the depressor nerve is the one excited. These facts may perhaps serve to explain to some extent the profound shock caused sometimes by abdominal and other injuries, if we believe shock to be due to a reflex dilatation of the abdominal vessels.

It is unnecessary to explain further why when these depressor effects are observed the kidney shrinks. This is of course due to the

dilatation occurring in a large area, and the kidney is affected secondarily. Finally, although no doubt the dilatation of any large vascular area would affect the kidney secondarily, yet it is probable that such dilatation in the vessels of the abdominal area would affect it most easily. Having thus described the reflex phenomena, we will now sum up shortly the various results obtained.

VI. Conclusions.

It will not be necessary to give here more than a bare summary since all the results have been discussed when described.

(*a*) All renal vaso-motor fibres leave the spinal cord, though anterior roots and although a few fibres may come out as high as the 4th dorsal, it is not until the 6th dorsal is reached that they are found in any abundance. From the 6th dorsal to the 13th dorsal they are abundant, below this they are found in rapidly diminishing numbers, so that but little vaso-motorial effect is seen to follow the excitation of the 3rd and 4th lumbar nerves.

(β) These renal vaso-motor fibres are of two kinds, vaso-constrictor and vaso-dilator. The former are, however, by far the best developed, so that unless special methods are used it is rare to get clear evidence of vaso-dilatation on excitation of any given nerve.

(γ) The kidney vessels receive their nerves from all the spinal nerves included in this extensive series, but most of the renal vaso-motor fibres are found in the 11th, 12th and 13th dorsal nerves.

(δ) No evidence has been obtained by me of the existence of any vaso-constrictor fibres for the kidney vessels in the vagus nerve[1].

(ϵ) By reflex excitation it is more common to get contraction than expansion of the kidney, but expansion is frequently obtained by excitation of the central end of a posterior root of a nerve belonging to what may be called the renal area, i.e. the 11th, 12th, or 13th. It is, however, not uncommon with the higher posterior roots.

On the other hand, excitation of the central end of an intercostal nerve produces contraction of the kidney, hence the expansion observed with the central end of a posterior root is apparently due to the stimulation of afferent visceral nerve fibres.

Occasionally reflex excitation produces a general dilatation, and

[1] I hope to enter into this question more fully in a subsequent communication on the secretion of the kidney.

then the kidney (although its vessels share in the dilatation) undergoes passive shrinking. Finally, there is no evidence to show that any decussation of the vaso-motor fibres in the splanchnic occurs, i.e. the right splanchnic nerve does not apparently supply the left kidney, but only the right.

The expenses of this research were partly defrayed by a grant obtained from the Royal Society.

LONDON, *April*, 1889.

EXPLANATION OF FIGS. 1—20. PLATES XXIII.—XXVI.

All the curves are to be read from left to right. All the blood-pressure curves were taken with a mercurial manometer connected with the carotid artery. The animals were curarized and the anæsthetics were either chloroform and morphia or chloroform alone. The tracings were all obtained from dogs unless otherwise specified.

The following abbreviations are used. B.-P. = blood-pressure. K = kidney curve. H = Helmholtz side wire to Du Bois coil. D = Daniell cells. P.R. = posterior root.

Fig. 1. Dog. Time tracing marks seconds. Oncograph. 7th dorsal nerve excited outside dura mater after division of posterior root. Coil 4 H. 1 D. i.e. secondary coil 4 centimeters from primary. Helmholtz side wire and 1 Daniell cell. This tracing shows rise of B.-P. and contraction of kidney, and after the cessation of the excitation the kidney curve shows some rhythmical contractions of the renal vessels.

Fig. 2. Same dog and same nerve as in Fig. 1 and same strength of stimulus. The renal vessels have become rigid during the excitation, but there is practically no diminution in the kidney volume although the rise of blood-pressure is considerable.

Fig. 3. Same dog and details as in Figs. 1 and 2, but in this case the kidney trace shows the rigidity of the vessels and a diminution in the volume of the kidney.

Fig. 4. Dog. Oncograph. 8th dorsal nerve (posterior root divided) excited outside dura mater. Slight expansion of kidney, no obvious rise of B.-P.

Fig. 5. Same dog as in Fig. 4. 10th dorsal nerve (posterior root divided) excited outside dura. Great contraction of kidney after a slight initial expansion and a persistent after-effect. Marked rise of B.-P.

Fig. 6. Same dog as in Figs. 4 and 5. 9th dorsal nerve (posterior root divided) excited outside dura mater. The renal contraction is not so marked as that seen in Fig. 5 with the 10th nerve, but it is followed by a "secondary shrinking" of the kidney accompanied by a very slight fall of B.-P.

Fig. 7. Dog. Oncograph. 10th dorsal nerve prepared and excited as in Fig. 5. The trace shows the rhythmical contractions of the renal vessels after the cessation of the excitation.

Fig. 8. Dog. Oncometer and piston recorder. 12th dorsal nerve, posterior root cut and nerve excited outside dura mater. This tracing shows the three results that can be obtained on nerve excitation. Firstly, a contraction of the renal vessels with a rise of B.-P. Secondly, a persistent contraction of the kidney with the B.-P. still above the normal. Thirdly, a "secondary shrinking" of the kidney accompanied by a slight fall of B.-P.

Time trace marks 2 second intervals.

Fig. 9. Dog. Oncometer and piston recorder. 13th dorsal nerve cut and ligatured. B.-P. shows Traube curves. Time trace marks 2 second intervals coil 6 1 D. Slow rhythmical shocks, i.e. 1 per second gives expansion of kidney and a very slight rise of B.-P. which is however only a better marked Traube curve than usual. Quick rates, i.e. 50 per second, give contraction of kidney and a great rise of B.-P.

Fig. 10. Same dog and same details as in Fig. 9. Renal expansion with slow rates still more marked and unaccompanied by any marked rise of B.-P.

Fig. 11. Dog. Oncometer and piston recorder. Expansion of kidney and increased visibility of cardiac pulsations on kidney trace during slow excitation of 13th dorsal nerve outside dura, after division of posterior root. Contraction of kidney with quick rates.

Fig. 12. Dog. Oncometer and piston recorder. Time trace marks 2 second intervals. Right splanchnic cut and ligatured peripheral end excited with slow shocks, i.e. 50 per minute. Slight fall of B.-P. and shrinking of the kidney after cessation of excitation. The same result is obtained during the excitation if this is of longer duration.

Fig. 13. Dog. Oncometer and piston recorder. Splanchnic nerve excited with slow rhythmical shocks, i.e. 50 per minute. Fall of B.-P. and shrinking of kidney.

Fig. 14. Rabbit. Oncometer and oncograph. Excitation of central end of depressor nerve. Great fall of B.-P. with comparatively slight effect on the kidney.

Fig. 15. Cat. Oncometer and oncograph. Excitation of central end of left vagus, right vagus having been previously divided.

Fig. 16. Dog. Oncometer and oncograph. Excitation of the central end of the posterior root of the 12th dorsal nerve. Expansion of kidney and rise of B.-P.

Fig. 17. Dog. Oncometer and oncograph. Excitation of the central end of the posterior root of the 9th dorsal nerve. Expansion of kidney and rise of B.-P.

Fig. 18. Dog. Oncometer and oncograph. Excitation of central end of the posterior root of the 11th dorsal nerve. Mixed effect on kidney with rise of B.-P.

Fig. 19. Dog. Oncometer and oncograph. Excitation of the central end, the 10th dorsal posterior root. Fall of B.-P. and shrinking of kidney.

Fig. 20. Cat. Oncometer and oncograph. Vagi divided. Excitation of central end of divided sciatic. Fall of B.-P. after a long latent period and accompanied by a considerable contraction of the kidney.

ON THE STRUCTURE OF THE VERTEBRATE LIVER[1].

By THOMAS W. SHORE, M.D., B.Sc., and H. LEWIS JONES, M.A., M.D. Plates XXVII.—XXIX.

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A. Preliminary.

This paper is put forward as a contribution to the knowledge of the structure of the mammalian liver, the subject being approached from the side of Comparative Anatomy. We have attempted, by examining a series of forms, to trace the steps between the undoubtedly tubular liver of the lower vertebrates and the apparently parenchymatous arrangement which constitutes the proper tissue of the lobule of the liver of the mammal. The structure of the liver in the lower vertebrata was the subject of a valuable paper by Eberth[2] in 1867, and since that time the liver in many vertebrated animals has been histologically examined by different observers, and its structure briefly described by them in various scientific journals. As however, there are several points which are still obscure, we think that a careful description, with drawings, of the minute structure of the liver in a number of widely separated vertebrated types may prove to be of service.

The literature on the subject shows, as might have been expected, that the problem of explaining the appearances seen in sections of the mammalian liver on the hypothesis that it is a modified tubular gland, has been several times taken in hand by histologists, but no satisfactory results have, so far as we can learn, as yet been arrived at. Lionel Beale[3], in describing his investigations into the structure of the liver in various vertebrata continually makes use of the phrase, "the tubes of the liver," and expresses his conviction that the liver is a tubular gland. We find in his paper the following important passage;—"The tubes (of

[1] Towards the expenses of this research, a grant was made by the British Medical Association, on the recommendation of the Scientific Grants Committee of the Association.

[2] Eberth, *Arch. f. mik. Anat.*, 1867.

[3] Lionel Beale, "On some points in the structure of the Liver," *Phil. Trans.* 1856.

the liver *sc.*) in birds contain two or three rows of cells and in the embryo mammal there are frequently two or three rows.....The similarity of the arrangement of the cells in the tubular network of the embryos of the higher animals with that existing in the adult as well as in the embryo of the lower animals is a point of interest." Beale also considers the question of whether a basement membrane around the secreting cells is present or absent in the liver, a point which we hope to show to be one of considerable importance. After quoting the names of Krukenburg, Van der Kolk, Retzius, Theile and others in support of the opinion that a basement membrane does exist, and the names of Handfield Jones and Kölliker in support of the opposite view, he proceeds to say that there is a basement membrane surrounding the rows of cells which constitute the tubules of the liver. Heidenhain[1] however after specially directing his attention to this point failed to find any basement membrane, and Klein[2] says that "the membrana propria of the bile ducts is not continued into the lobule on the surface of the liver cells as is maintained by Beale, Pflüger and others." Hering[3] in his account of the structure of the liver quotes von Biësiadecki to the effect that the human liver is a tubular gland like that of the snake with a lumen surrounded by five rows of cells, but he adds;—"I have not seen anything of this kind even in the liver of the new-born child, which as contrasted with that of adults does exhibit some similarity in structure to that of amphibia, as for example the frog, inasmuch as it frequently presents three or four cells enclosed in the rounded meshes of a capillary plexus whilst they themselves again form the parietes of a minute biliary canal." Hering also figures the liver of a snake to show that it consists of a system of anastomosing gland tubules interlaced with anastomosing capillary blood-vessels, and he discusses at length the possibility of two such anastomosing networks coexisting in one organ. Heidenhain[4] copies this figure and gives several diagrams or schemes of the mammalian liver arranged as a network of densely packed anastomosing tubules. He lays down the law that every liver cell has a blood channel on one side and a bile channel on the other, the bile channels never being in direct contact with a blood capillary, but always having

[1] Heidenhain, in Hermann's *Handbuch der Physiologie*, 1883.

[2] Klein and Noble Smith, *Atlas of Histology*, p. 230.

[3] Hering, Art. on the Liver. Stricker's *Manual of Histology*, 1867. English translation.

[4] *loc. cit.*

a liver cell lying between them. He further says;—"The bile channels generally run between the flat sides of contiguous cells, less often along the angles where three or four cells meet, but only if no blood capillary occupy that angle." He adds that Hering at first believed from examining the liver of rabbits only, that the position of the bile channel on the flat surfaces of the cells was its sole one; but later he found that in dogs and in man it is also situated at the angle, and he quotes Peszke[1], who states that this latter position of the bile channel is as frequently found as the other. Heidenhain quotes Eberth[2] in support of his statement that the liver of the bird is comparable to that of the reptile. Eberth's paper is illustrated by drawings of the microscopic appearances of the liver in *Cyprinus*, *Triton*, *Gallus*, and one or two other types. His drawing of the liver of *Cyprinus* shows it to bear a very close resemblance to that of *Muræna* which we describe and figure in this paper. His figure of the liver of *Gallus* though somewhat diagrammatic seems to afford a good idea of its structure; and it is evident from his drawings of the liver of *Triton* that, in interpreting its structure he has experienced the same difficulty as has been felt by ourselves and others. Eberth also describes and delineates the layers of cells to which we shall allude in our description of the liver of Cœcilia under the name of a "pseudo-capsule," and he mentions the interesting fact that these cells possess the power of amœboid movement during the life of the animal.

Barfurth[3] has described and figured the liver of Gasteropods as made up of a series of branched ducts terminating in cœcal enlargements, lined by large cells. This however is foreign to our subject, for though we can confirm his account of the liver of *Helix pomatia*, we have not yet attempted to trace any structural connexion between the so-called "liver" of invertebrates and that of vertebrata.

In the course of our investigations we have made as complete an examination as possible into the structure of the liver of the following vertebrated animals;—

PISCES—Lamprey (*Petromyzon*), Dogfish (*Scyllium*), Skate (*Raia*), Eel (*Muræna*), Flounder (*Pleuronectes*).

AMPHIBIA—Frog (*Rana*), Newt (*Triton*), Cœcilian (*Cœcilia*).

REPTILIA—Snake (*Pelias*), Tortoise (*Testudo*).

[1] Peszke, "Beitr. zur kenntniss der fein. Bau. d. Wirbelthierleber," *Diss. Dorpat*, 1874.

[2] Eberth, *Arch. f. mik. Anat.* xxxix. 1867, s. 70.

[3] Barfurth, *Arch. f. mik. Anat.* 1883, p. 473.

AVES—Pigeon (*Columba*), Fowl (*Gallus*).

MAMMALIA—Rat and Mouse (*Mus*), Mole (*Talpa*), Pig (*Sus*), Cat (*Felis*), Dog (*Canis*).

We have made use both of mature individuals and also in some cases of embryos.

The method generally followed in the preparation of the specimens has been after hardening, to stain *en masse* in cochineal, and to imbed in paraffin, afterwards cutting with a rocking microtome. The tissues have been hardened either in alcohol of gradually increased strength, or in Muller's fluid or in the chromic acid and spirit mixture recommended by Klein, the best results perhaps having followed the first of these methods. The cochineal stain, which has proved very useful by reason of its good penetrating and selective powers, is prepared as follows;—Take seven grammes of cochineal and seven grammes of alum finely powdered, and thoroughly rub them together in a mortar; add seven hundred cubic centimetres of distilled water, evaporate down to four hundred, filter twice and afterwards add four cubic centimetres of absolute alcohol.

Small pieces of most of the material have been hardened and stained in 1 p.c. solution of osmic acid, and by this means several valuable specimens have been obtained. This reagent has the well-known effect of staining the granules in the liver cells and has therefore served to bring out into clearer view the position and course of an otherwise indistinct lumen. As the number and situation of the granules in the liver cells is to a certain extent dependent upon the condition of starvation or otherwise of the animal[1], and also to some extent on the stage of digestion, we wish it to be understood that this method is only used in a general way and without special regard to these differences. We have also in a few cases cut sections with Williams' freezing microtome and stained them with logwood. To some extent also, we have availed ourselves of the method of injection of the blood-vessels and bile capillaries.

B. DESCRIPTIVE.

We now proceed to an account of the minute structure of the various livers examined, adopting the following order of description in each case;—

[1] Langley, "On changes in the liver cells under various conditions." *Proc. Roy. Soc.* 1882.

1. The cells, their size and appearance, and the general arrangement of the intra-cellular granules when they seem to call for special remark. We have not deemed it advisable in all cases to state the exact measurements of cells, as we find that the semidiameter of the tubule affords the best means of comparing the relative size of the liver cells in different animals, the lumen being in most cases of inappreciable diameter under ordinary powers of the microscope.

2. The number of rows of cells making up the walls of the tubules.

3. The compactness or otherwise of the organ; the arrangement, convolutions and anastomoses of the tubules, and the situation of the larger ducts.

4. The inter-tubular blood channels, and the other blood-vessels.

5. Other points of importance or interest.

I. PISCES. In all the fishes which we have examined the liver is made up of a network of anastomosing tubes with small lumina, and, with the exception of the lamprey, the general plan of structure is obviously the same in all. In *Petromyzon*, a type of the lowest group of vertebrates possessing a liver in the ordinary sense of the word, there are such wide differences from what is seen in the remainder of the class that we shall describe it separately; and though in all probability it represents an earlier step in the evolution of the liver than that of other fishes, yet we shall describe it after we have dealt with that of the more typical members of the group. The structure of the liver in many fishes is obscured by the presence of large oil drops in the cells, but the liver of the eel is free from this objection. We will therefore first describe it and afterwards briefly mention any points in which that of other fishes differs from it.

1. *Muræna.* The cells of the eel's liver are of medium size, and are granular throughout with a condensation of granules towards the lumen, which is distinct though small. The number of rows of cells forming the wall of the tubule is generally five or six, but in some cases is four. The diameter of the tubule averages 25μ, but is not quite uniform (Plate XXVII. Fig. 2). Tubules may easily be traced in the plane of the section for a distance of from twelve to fifteen cell diameters, particularly where they show a roughly radiating arrangement round a large blood-vessel. They branch and anastomose on an average at distances of about six to eight cell diameters when seen as they lie in the plane of the section; but the meshwork is closer where the section passes transversely through it, an appearance which leads one to infer that the meshes of the network are elongated, and have a long diameter three or

four times that of their shorter one. The smallest ducts, including their walls, measure about 20μ across; they are few in number in any section, and consist of three or four columnar cells surrounded by a membrane made up of a small amount of connective tissue. The larger ducts have a thick fibrous coat and lie near the larger blood-vessels. The blood channels form a network of irregular spaces, which are of varying calibre and have a delicate wall of endothelial cells. No basement membrane is to be made out separate from the wall of the capillary blood-vessels. A well-marked radial arrangement around the larger blood-vessels of both the finer blood channels and the anastomosing liver tubes is found in some parts of the section, and here and there it can be seen that the capillary spaces run directly into these central vessels, but this is not the case with more than a small minority of the whole number, for in some, and these the largest central trunks, the radial arrangement is plain, but no capillary can be seen to enter the central vessel. The periphery of these radially arranged systems of tubules and blood capillaries is quite indefinite, and there is no true division of the organ into lobules at all comparable to those of the liver of the Mammal; and further we are not certain whether these central trunks belong to the portal or the hepatic system of veins, nor is the question simplified by the uniform presence or uniform absence of a bile duct running with the vessel; for the most part however there is no such duct and these vessels would therefore seem to be radicles of the hepatic vein. Here and there in the section, are found oval masses of an orange pigment of varying size, situated generally by the side of a large blood-vessel, and having, so far as can be made out, no investing wall of epithelium. These are probably masses of bile pigment, either extravasated or encysted along the lines of the bile ducts.

2. *Pleuronectes.* The liver in the flounder and similar fishes is in minute structure very like that of the eel. The flounder's liver is more compact, and therefore its general appearance with a low power is more like that of a mammal than is that of the eel, but the radial arrangement is less perceptible. Some of its blood-vessels have very thick walls containing much muscle, whilst others of the blood-vessels have not these thick walls, and as the bile ducts lie near these latter they are probably branches of the portal vein.

3. *Scyllium.* The liver tubules in the dog-fish are composed of larger cells than those of the eel. The cells are very full of oil drops, and the disposition of their nuclei is irregular, being apparently displaced by these oil globules, which are so numerous as to leave merely a loose

network of protoplasm to form the cell. Each tubule measures from 50μ to 70μ in diameter, and the anastomoses are less apparent than in the liver of the eel. The blood channels have well defined thick walls of endothelial cells and they form a network which is more conspicuous in proportion as the network of tubules is less so. The bile ducts have the usual structure and run with the larger blood-vessels, around which no radial arrangement of the tubes or blood capillaries is found.

4. *Petromyzon.* The liver of the lamprey differs remarkably from that of all other vertebrate types which we have examined. In the first place the liver cells are very small, and secondly there are no tubules such as are found in the liver of other animals. Our first impression was, that there were in this liver large lumina with cells grouped round them though not in a single layer; but further examination made it clear that these supposed lumina are really blood channels and that between these blood channels the liver cells are disposed in solid anastomosing cylinders, the central cells of which are loosely arranged, with intercellular spaces occupying what may be viewed as the lumen of a potential tube. (Plate XXVII. Fig. 1.) The relative proportion of cell-cylinder to blood-vessel is extremely large, and between any two blood-vessels the number of layers of cells varies from two to five or six. The cells of which the intervascular cylinders are composed naturally fall into two classes, distinguished by their form and arrangement;—(*a*) those immediately adjacent to the blood-vessels, which have the form and arrangement of a columnar epithelium disposed radially round the blood channels, and each measuring 5μ in width and about 15μ in length, (*b*) those situated within the cell-cylinders, which are generally polyhedral in form, some few being slightly elongated and each measuring about 5μ in all diameters. The latter are disposed in a spongy manner and in hardened specimens show channels running between them. In all the cells, the nuclei stain lightly, but the nucleoli are very distinct. The protoplasm is uniformly granular. Between the surfaces of the cylinders of cells and the walls of the blood-vessels there is no basement membrane. The liver of the lamprey may be described as a mass of cells permeated by a network of narrow capillary blood channels roughly dividing the organ into an anastomosing network of broad cylinders. Around the larger blood-vessels the cell cylinders are disposed in a radiate manner. We are unable to find any small ducts.

II. AMPHIBIA.

1. *Rana.* The cells of the frog's liver are large and clear. They have granules on their inner borders, next the lumen, which feature is brought out very clearly by treatment with osmic acid, but is also visible in good logwood-stained specimens, one of which is figured in Plate XXVII. Fig. 4. Langley[1] states that in summer a hungry frog has granules equally scattered throughout the cells of its liver, and that during winter there is a marked inner granular zone. We can confirm this, and the specimen figured in Plate XXVII. Fig. 3, is from a winter frog, which on account of the distribution of the granules is the one best suited for tracing a lumen. In an osmic acid preparation the anastomosing liver tubules are rendered very plain by the darker staining of the blood in the capillaries, and they have the appearance of a lightly stained mass with the division into cells not very obvious, but with a well marked inner zone of black granules. In a logwood or cochineal-stained specimen the number of rows of cells is seen to be usually five. The tubules are more convoluted than those of the eel and they anastomose with greater frequency, it being rarely possible to trace a tubule in one plane for a greater distance than six cell diameters. In livers which have not greatly shrunk in hardening the structure has a very compact appearance, the blood-vessels being uniformly narrower than the tubules. The average measurement of the tubule is about 50μ. No basement membrane surrounding the tubules can be seen and there is no sign of any lobular arrangement. Small bile ducts are found running near the larger blood-vessels and have the usual structure.

2. *Cœcilia*[2]. The liver of a Cœcilian (Plate XXVIII. Fig. 5) is a good example of a well-developed tubular liver. In this creature, the liver consists of a series of foliaceous lobes, each three or four millimetres across, arranged in an imbricated manner along one side of a main duct, which sometimes is over two inches long. The cells are clear, or very faintly granular, with their nuclei arranged peripherally. There are five rows of cells lining the tubules, whose diameter varies considerably, the average size being 40μ. The lumen, though small, is very distinct, and there is no basement membrane. The tubules are arranged in elongated meshes, are much convoluted, and the anastomoses are not very abundant. The relative diameter of blood capillaries when compared with that of the tubules is greater than in the case of the frog. The blood-vessels

[1] Langley, *loc. cit.*

[2] We are indebted for this material to the kindness of Dr Frank Penrose.

are irregularly arranged and show no sign of being radially placed, but are seen to be somewhat elongated in the plane of the flattened lobes. The larger vessels have very thick walls and are situated with the bile ducts at the margin of the lobe. Each lobe is enclosed in a sort of pseudo-capsule formed by a condensation of the cells near the surface and having a diameter of from 25μ to 50μ. This layer of condensed cells lies beneath the true fibrous capsule. There is a similar condition in the liver of the newt, but in no other animal which we have examined[1].

3. *Triton.* The liver of the newt is very compact and its tubular structure is not very apparent. We have not succeeded in securing a good natural injection of its blood-vessels and this has rendered the tracing of the tubules more difficult. The individual cells are large, measuring 25μ in diameter in a specimen fixed in osmic acid, and have large nuclei, 10μ to 12μ in diameter. The cells have a general granular appearance slightly more marked at their margins, and not confined to the neighbourhood of the lumen. There is a considerable number of large granules which blacken in osmic acid and are irregularly scattered throughout the cells. Langley[2] found that the granules in the liver of the newt are large and show clearly that they are not the nodal points of the intra-cellular network. He observed no arrangement of the granules into zones during digestion and he says;—"The newt's liver appears to me to depart largely from the tubular type of gland and to resemble in structure rather the mammalian than the ordinary amphibian liver." Our observations lead us to agree in the main with this statement, but though indistinct the tubule can nevertheless be discerned. The tubule measures about 50μ in diameter and is composed of three or four rows of cells surrounding the lumen. The intertubular blood channels are narrow, their diameter being about equal to that of a newt's blood corpuscle. Here and there, a short double row of cells, suggesting a length of straight tubule, can be traced for a little distance in the plane of the section. There is no basement membrane between the capillary walls and the secreting cells. The newt's liver is essentially a parenchyma of large cells, permeated by a network of fine blood capillaries and drained by a reticulum of minute bile channels, which lie usually at the angles where three or four cells meet, and so arranged that each cell has on some side of it a blood capillary and is connected at one point, at least,

[1] Eberth, *loc. cit.*, described the same condition in *Proteus*.
[2] Langley, *loc. cit.*

of its surface with a bile channel (Plate XXVIII. Fig. 6). Scattered through the organ are numerous rounded masses of a dark brown pigment, of irregular sizes, but having an average diameter of about 25μ. Similar pigment masses occur in the liver of the frog, of the Cœcilian and of the tortoise.

III. REPTILIA.

1. *Testudo.* The liver of the tortoise is the most beautiful of all the tubular livers that we have seen. It is composed of large cells having a clear outer zone and a dense collection of reddish granules forming an inner zone around the lumen which but for this fact would not be very conspicuous[1]. The nuclei lie near the periphery of the cells, four to six rows of which form the tubule. The tubule has a diameter varying from 50μ to 70μ, and is much convoluted with frequent anastomoses (Plate XXVIII. Fig. 7). The blood channels are of large, but very varying, calibre, some measuring as much as 100μ, and are enclosed by a nucleated endothelial wall which is adapted to the irregular surfaces of the tubules around them. Apart from this endothelial wall there is no basement membrane. There is no indication of a lobule. The ducts occupy their usual situation beside the larger blood-vessels, have the usual structure and are not very numerous. The tortoise's liver contains pigment masses resembling those of the newt, but larger. The organ is enclosed in a thin fibrous capsule.

2. *Pelias.* The liver of the viper is in the main very like that of the tortoise, but its cells and its tubules are smaller. The average diameter of the tubule is 40μ, and the walls of the blood channels are thicker than in the tortoise. The cells do not present the marked outer clear zone and the condensation of granules around the lumen which is seen in the tortoise, but are more evenly granular. Hering[2] figures and describes the liver of another snake (*Natrix*). Our preparations of the liver of *Pelias* confirm the accuracy of his drawing and description. We agree with him in finding a network of anastomosing liver tubules interlacing with a reticulum of capillary blood-vessels. The nuclei of the cells are near the periphery of the tubule, as in his drawing, and we also agree with it in finding that there is no basement membrane around the gland tubules.

IV. AVES. The liver of birds is extremely dense and the outlines

[1] We are unable to specify the exact condition as to hunger or state of digestion, in which this appearance is found.

[2] Hering, *loc. cit.*

of the cells obscure; and in sections of the liver of a full grown pigeon but little of the intimate structure can be seen in consequence. In young chickens, however, the arrangement is clearly tubular with five rows of cells to the tubules which have a diameter of 30μ to 40μ. The tubules are convoluted and anastomose frequently so as to form a close network, and the lumina are distinct. In the specimen figured in Plate XXVIII. Fig. 8, the cell protoplasm is completely honeycombed by a number of clear spaces filled with oil globules. The blood capillaries are small and there is an uniform distribution of larger vascular trunks, around which there is a tendency to a radiating arrangement, but no distinction into interlobular and intralobular veins can be seen.

V. MAMMALIA. The liver of an embryo cat measuring 15 m.m. in length bears a striking resemblance to that of the lower vertebrates and may well be compared with the liver of the eel. It is composed of a network of anastomosing tubules measuring 30μ in diameter with five rows of cells around a small lumen. (Plate XXIX. Fig. 9.) The nuclei lie almost centrally in the cells. The general arrangement is very irregular and the blood spaces are large. There is no basement membrane, though the walls of the blood capillaries are well developed, and there is no appearance of a lobule.

The general structure of the adult mammalian liver is so well known that any detailed description of it is unnecessary. Our own observations confirm the descriptions ordinarily given. In sections of a mammal's liver the bile capillaries of which have been injected the closeness of the meshwork of the bile channels can be well seen and is a striking feature of difference between the liver of these types and that of lower forms. According to Hering[1] and Eberth[2] the meshes of the network of bile capillaries are of the size and shape of the liver cells; and bile capillaries are never present between liver cells and blood capillaries (Hering, Heidenhain[3] and Peszke[4]). The situation of the bile capillaries in respect to liver cells can be well seen in osmic acid preparations, and is found in *Talpa* to be between either three or four cells (Plate XXIX. Fig. 11) and in *Mus* to be between three cells or less commonly only two (Plate XXIX. Fig. 10). A membrane around the bile capillaries, internal to the liver cells, is said by Asp[5], Peszke and

[1] Hering, *loc. cit.*
[2] Eberth, *loc. cit.*
[3] Heidenhain, *loc. cit.*
[4] Peszke, *loc. cit.*
[5] Asp, *Ber. d. sächs. Ges. d. Wiss. Math. physik. Cl.* 1873.

others to be present, but is denied by Hering, Eberth, Kolliker and others. We have not found a membrane in this situation.

We have not been able to satisfy ourselves of the arrangement of granules in the cells of the mole's liver as described by Langley[1] who states that in digestion there are rows of peripherally arranged granules giving almost the appearance of a cell wall. In our preparations, though the outlines of the cells are very distinct, the granules are uniformly distributed through them. In a cochineal-stained section of the liver of the pig the lobule is very distinct and the penetration of it by blood capillaries is extreme. The lumina of the bile capillaries in the pig's liver are not evident, but their positions can be readily seen by the accumulation of yellow coloured (bile) pigment at adjacent positions in contiguous cells (Plate XXIX. Fig. 12), and from this alone we are able to infer that the network of bile channels is a very close one, and lies between either two or three cells. Hering[2] figures the liver of a young child with a bile capillary lying at the angle between three cells, and in the same figure another one lying between two cells is shown. In none of his figures is a basement membrane depicted, and in his description he is not satisfied that there is one distinct from the capillary wall. Heidenhain[3] also has failed to find such a membrane, nor can we find anything of the sort in the pig's liver.

C. Theoretical.

The most striking feature brought out by an examination of the series of vertebrate livers above described is that this organ is in the lower vertebrates essentially a meshwork of anastomosing tubes and that its structure is remarkably uniform in all, from the selachian fishes upwards through the amphibia and reptiles to the birds. It might have been expected that somewhere low down in the scale a liver would be met with consisting of tubes, convoluted but not anastomosing to form a network, and that in tracing it upwards an increasing complexity might have been discovered. The fact that no liver has been found with these characters, but that a meshwork of tubules is present even in the lowest types, raises the question whether the liver may be regarded as having sprung from a gland whose secreting tubes do not anastomose or whether it may not have arisen in some other way. The adult mammalian liver

[1] Langley, *loc. cit.*
[2] Hering, *loc. cit.*
[3] Heidenhain, *loc. cit.*

shows by the arrangement of its bile capillaries that its meshwork is much closer than in any other vertebrate, (though in this respect the liver of the newt somewhat approaches it,) and that the change through which it has passed in its evolution from that of some lower type is in the direction of increased production of secretion channels with the formation of a finer anastomosis. A tubular liver of the type of that of the eel, but with an extremely close meshwork of bile capillaries and with a reduction of the number of cells surrounding the lumen from five to three or to two would present very nearly the appearances seen in a section of a mammalian liver; where the bile capillary occupies the angle between three cells it would represent a tubule lined by three rows of cells, and where it is situated on the flat surface between two cells, then the tubule would be one having but two rows of cells around its lumen. One might, then, suppose that the mammalian liver was evolved from one like that of the fishes by an increase in the frequency of anastomoses between tubules, with a diminution in the number of rows of cells lining them; and that this reduction of cells coupled with the increased condensation of the gland had rendered it difficult to recognise in the mammalian liver the tubules of which it is built up. This hypothesis as to the mode of formation of the mammalian liver is supported by the accepted description of the development of this organ. Balfour[1] after describing the origin of a diverticulum or diverticula from the duodenum says:—"From the primitive diverticula there are soon given off a number of hollow buds, which rapidly increase in length and number and form the so-called hepatic cylinders. They soon anastomose and unite together and so constitute an irregular network. Coincidently with the formation of the hepatic network the united vitelline and visceral vein or veins in their passage through the liver give off numerous branches, and gradually break up into a plexus of channels which form a secondary network amongst the hepatic cylinders." It is not till quite late that the irregular blood channels assume the arrangement found in the hepatic lobules of the adult. If we assume, as would be justified by Balfour's account of the actual development, that a fusion of the extremities of originally distinct branches of a tube can occur, then it is easy to understand how by a repetition of the same process a meshwork of any degree of closeness may arise. In support of the possibility of the occurrence of such anastomoses one would look for analogous processes elsewhere in the body. In the course of the forma-

[1] Balfour, *Comparative Embryology*. Vol. II. p. 632.

tion of the vertebrate excretory system the extremities of the segmental tubes unite with the previously formed segmental duct; and it is believed by Lockwood[1] that the developing testis tubules become united at their extremities with the ends of those tubes of the Wolffian body which are about to form vasa efferentia. In the development of capillary blood-vessels, union together of mesoblast cells to form a network of tubes is well known. On the other hand, no such fusion of the ends of tubes is seen in the course of the development of the pancreas although it arises at about the same time as the liver and, like it, as an outgrowth from the gut. Nor is such an anastomosis known in the adult structure of any other tubular gland. It is difficult to see why such should occur in the case of the liver only. We find it difficult to believe that union of the ends of distinct tubes like those of, say, the salivary glands or pancreas, provided as they are with a basement membrane, could take place, but this is more easily conceived possible in the case of a gland having no basement membrane. The presence or absence of a basement membrane in the liver is a disputed point. As we have previously mentioned, several observers have thought that such a membrane was present, whilst others, and notably Heidenhain and Klein, deny its existence. Our investigations have failed to discover in the liver of any type a trace of such a membrane apart from the walls of the blood capillaries.

Although the "liver" of some invertebrata consists of a series of cœcal tubes, yet the absence, even in the lowest vertebrates, of a liver without meshes certainly suggests the belief that the network of tubules in the vertebrate liver is present *ab initio* and is not derived from one without anastomosing tubes. If the meshwork has not arisen by the fusion of originally distinct cylinders of cells as described by Balfour and other embryologists, it seems to us that there is but one other way in which it could have had its origin, viz.:—from a solid mass of glandular cells, by the development within it of a network of intercellular spaces. We venture to put forward this view as a new explanation of the uniform presence of anastomosing tubes in the vertebrate liver.

There is nothing in the known history of the development of the liver which opposes the idea that the gland may have arisen from a solid mass of hypoblast cells formed at the extremity of the primitive hepatic diverticulum and which growing and forcing its way into the surround-

[1] Lockwood, "Development and Transition of the Testis." *Hunterian Lectures*, 1888.

ing mesoblast, becomes itself interpenetrated by ingrowing mesoblast cells. By the development of these latter into blood capillaries the hypoblastic cell mass would become broken up into a network of solid anastomosing rods, in which secretion channels would subsequently form, and which would be more or less fine according to the completeness with which the ingrowth of developing blood-vessels took place. It is still doubtful whether the hepatic cylinders are at first hollow or solid. Balfour states that they are hollow in elasmobranch fishes, in amphibia and in some mammals. In *Aves* both Remak and Kölliker find them to be at first solid, as is the case also, according to Kölliker, in the rabbit.

Such an explanation as this would account for, nay would demand, the existence of an anastomotic network even in the simplest vertebrate livers, and would explain the appearances found in the more specialised liver of mammals as being the result of a more complete penetration by blood-vessels and a greater subdivision of the mass of secreting cell elements, so that the bile capillaries, instead of having as in the lower vertebrates four, five or six rows of cells around them, are bounded by three or even only two cells. Just as the less distinctly tubular liver of the mammals as compared with the prevailing type found in fishes, amphibians and reptiles would be due to a more complete penetration of blood-vessels, so would the apparently anomalous liver of the lamprey be explained as having resulted from a very imperfect ingrowth of blood capillaries, so as to leave between the capillary blood meshes relatively large masses of hepatic cells amongst which but a very indefinite formation of biliary channels has taken place[1].

In a section of the liver of any of the types we have examined it is remarkable how extremely few small, or even medium sized, bile ducts are cut across, and in all cases whatever ducts are present have well marked walls of connective tissue lined by an epithelium quite different in character from the cells composing the liver tubes. In the case of the liver of the lamprey we failed to find any small ducts and in that of *Cæcilia*, there seems to be only one very large main duct running with the portal vein along one side of the organ and apparently receiving minute communications along its whole course from the liver tubules of

[1] Wiedersheim (*Comparative Anatomy of Vertebrates*, Engl. transl. p. 242) states that the liver of the lamprey is degenerate, but specifies no grounds for this opinion, which is possibly founded on the current belief that in the adult lamprey the opening of the bile duct into the intestine has closed up. By some biologists the lamprey is believed to be degenerate, but we agree with Bateson ("Ancestry of the Chordata," *Q. J. Micr. Science*, 1886) in failing to find any ground for this opinion.

which each foliaceous lobe is made up. These facts suggest that the primitive hepatic diverticula alone have developed into bile ducts, and that the solid cell mass formed at their extremities and from which the proper liver tissue is developed, is to a great extent distinct from them. They also suggest that the bile capillaries are not formed by an extension of the lumen of the primitive diverticulum but rather by the production of finer channels in the midst of solid rods of cells; and that the extent to which, with the growth of the organ, the branches of the primitive diverticula have penetrated into the proper liver substance, so as to form fine ducts, has been but limited. The way in which the bile capillaries terminate in the finest bile ducts at the periphery of the lobules has been described and figured by Asp[1] and by Klein[2]. In the liver of the mammal, fine bile ducts are only very rarely found within the lobule, but we have in few instances seen them, especially in the liver of the pig. In relation to this part of the subject it is interesting to note that Kiernan[3] in 1833 figured an anastomosis between branches of the bile duct in the triangular ligament of the human liver, and that E. H. Weber[3] described ducts (vasa aberrantia) which formed a network in the transverse fissure uniting the right and left hepatic ducts. Beale also, and others have shown that the smallest bile ducts form an interlobular anastomosis amongst themselves.

An examination of the liver of the types we have described in the light of our hypothesis will show that all the appearances can be explained by it. We can find no other satisfactory interpretation of the arrangement seen in the liver of the lamprey (Plate XXVII. Fig. 1). A comparison of a section of the eel's liver with that of a mammal (Plate XXVII. Fig. 2 and Plate XXIX, Fig. 12) shows clearly that a more intimate subdivision by capillaries in the case of the latter would account for the differences, and at the same time it is seen that the relative magnitude of the blood capillaries in the two cases is very different—those of the eel can scarcely be called "capillaries". That this is the true difference in the two cases is clear from a comparison of a section of the liver of an embryo mammal with that of the adult and with that of the eel (Plate XXVII. Fig. 2, and Plate XXIX., Figs. 9 and 12). On examining a section of the liver of the newt, the appearances seen, making allowance for the different sizes of the cells and smaller details, are more like those of the mammal's liver than that of any other of our types—(Plate XXVIII. Fig. 6). There has been in this case a pene-

[1] Asp, *loc. cit.* [2] Klein, *loc. cit.*
[3] Quoted by Beale, *loc. cit.*

tration of the mass of liver cells sufficiently intimate to have left but three or four rows of cells to form the tubules, and at the same time the total vascularity of the organ, as shown by the size of the blood spaces, is not so great as in the case of the eel or the mammal. The absence of a basement membrane around the liver tubules is on our hypothesis explained by supposing that the growth and production of blood capillaries in the original solid mass of liver cells was not accompanied by the formation of ordinary connective tissue elements. On the other hand the presence of connective tissue in the walls of even the smallest ducts is explained by the supposition that they are solely formed from the primitive hepatic diverticulum which carried with it developing connective tissue from the wall of the gut. The arrangement of the bile ducts within the organ, and their scarceness, is also satisfactorily explained.

Perhaps however the most characteristic feature in the mammalian liver is the subdivision of the organ into lobules, a condition which is uniformly absent in the liver of the other types we have examined. In endeavouring to explain the lobule, one would naturally seek, in lower forms, for any trace of its most striking character, viz.:—the presence of capillaries and rows of liver cells or liver tubules more or less radiating round a small central vein. It will be noted that in several of the cases which we have described such a radiating arrangement of an imperfect and indefinite kind is seen, but in all these cases the radial appearance is not found around a small vessel such as would form a radicle of the hepatic vein, but is in the proximity of a *larger vessel* and one which has thick and definite walls and into which the indefinitely radiating capillaries cannot as a rule be traced. Nor in these cases do we find any peripheral outline however indistinct mapping out the borders of these radiating systems. These cases are not, we think, instances of incipient lobulation. We imagine that the lobule has arisen, during the growth of the organ, by the formation in the network of blood capillaries of foci of exit for the blood and by the consequent tendency for the plexus of fine vessels to converge to these points. In some mammals the formation of the lobule has progressed but little beyond this condition, for sometimes the margins of the lobules are, in the absence of injection, only very indefinite. Following upon this condition there appears to have taken place a more complete and intimate ingrowth of bile ducts and branches of the portal vein, carrying with them more or less connective tissue from the porta of the liver so as to form a Glisson's capsule. In this way we imagine that the outlines of the lobules have become definitely mapped out. The extent to which this has progressed in different

mammals is different; in the majority it appears to be fairly marked, but in some, notably the camel and the polar bear, it has attained to a high degree of perfection. Turner[1] found in the camel that not only is Glisson's capsule well marked, but that there is in the peripheral part of the lobule a sustentacular connective tissue framework quite distinct from the network of capillary blood-vessels; but he was unable to determine whether any relation existed between this framework and the intralobular biliary passages.

To sum up. We can picture the vertebrate liver as having been evolved by some such steps as the following:—

1. The formation from the gut of a hollow diverticulum constituting at first a simple tubular gland lined by specially modified secreting endoderm. This stage is represented in actual anatomy by the simple hepatic diverticulum of the *Amphioxus*, and is reproduced in the ontogeny of the higher vertebrates by the formation of the primary hepatic outgrowth of the hypoblast, as in the chick, &c.

2. The copious subdivision of the endoderm cells at the cœcal extremity of this diverticulum to form a solid mass of cells, slightly penetrated by minute channels for escape of secretion, the original diverticulum becoming a duct.

3. The further multiplication of the cells of this mass and its penetration by blood-vessels so as imperfectly to divide it into solid anastomosing rods of secreting cells drained by a system of intercellular bile canals. This step is probably preserved in the liver of the lamprey.

4. The more complete penetration of blood-vessels between the cylinders of hepatic cells so as to form a well-marked system of broad blood channels, separating a network of rods of cells which come to be arranged in a single layer round bile capillaries. This is seen as the permanent condition of fishes, amphibians and reptiles, and is ontogenetically repeated in the development of the mammal.

5. A still finer penetration by blood-vessels, and further subdivision of the hepatic cylinders associated with an arrangement of the blood capillaries around foci of exit for the blood (hepatic vein radicles), so as to form hepatic lobules. This is the condition found in the adult mammalia.

[1] Turner, "Camel's Liver," *Journ. of Anat.*, 1877.

D. Conclusions.

1. There is no evidence in any type of vertebrates that the gland tissue of the liver is, when fully formed, anything other than an anastomosing network of tubules.

2. There is no evidence of change of plan as we ascend the vertebrate scale.

3. We have found no evidence that the network has been formed by the fusion of originally distinct tubules.

4. The liver is originally a solid mass of cells which has become penetrated by blood-vessels, and thus divided into a network of anastomosing cylinders.

5. The peculiarities of the liver in different types depend upon the extent to which blood channels have penetrated and subdivided it.

6. The special characters of the mammalian liver are explained by (*a*) a greater penetration of blood-vessels between the secreting cells, with their subsequent arrangement around foci of exit for blood; and (*b*) a coincident growth of connective tissue along the portal branches so as to map out the organ into lobules, a process which in different mammals has attained to varying degrees of perfection.

EXPLANATION OF PLATES XXVII.—XXIX.

Fig. 1. Portion of a section of the liver of the Lamprey. (× about 400 diam.)

a. Blood capillary with nucleated endothelial wall.

b. Blood capillary cut transversely. Around it is seen a radial arrangement of the elongated liver cells.

c. Solid hepatic cylinder with loosely arranged cells.

Fig. 2. Part of a section of the liver of the Eel. (× 400 diam.)

a. Transverse section of a larger vessel (probably radicle of hepatic vein).

b. Liver tubules cut transversely.

c. Lumen of a liver tubule.

d. Liver tubule in longitudinal section.

e. Nucleated endothelial wall of a capillary blood space.

Fig. 3. Section of an osmic acid preparation of the liver of the Frog. (× 400 diam.)

a. Blood corpuscles lying in a capillary vessel.

b. Outer lightly stained zone of the cells of the liver tubules.

c. Inner black-stained granular zone around the lumen of the tubules.

Fig. 4. Section of a logwood stained preparation of the liver of the Frog. (× about 400 diam.)

a. Sections of the network of spaces for blood capillaries.

b. Liver tubules in transverse section.

c. Lumina of liver tubules cut longitudinally.

Fig. 5. Section of the liver of *Cæcilia*. (× 400 diam.)

a. A blood capillary.

b. Section of a liver tubule.

c. Lumina of liver tubules cut transversely and longitudinally.

Fig. 6. Section of the liver of the Newt. (× 400 diam.)

a. Spaces for capillary blood-vessels.

b. Liver cells.

c. Bile capillaries.

d. Mass of brown pigment.

Fig. 7. Section of the liver of the Tortoise. (× 400 diam.)

a. Blood capillary containing clotted blood.

b. Endothelial wall of a blood capillary.

c. Inner granular zone of the liver cells.

d. Outer clear zone of the liver cells.

Fig. 8. Section of the liver of a young Chick. (× 420 diam.)

a. Capillary blood-vessel.

b. Endothelial wall of blood capillary.

c. Liver tubules cut transversely.

d. Lumen of a liver tubule.

Fig. 9. Section of the liver of an Embryo Cat, 15 mm. in length. (× 400 diam.)

a. Capillary blood space.

b. Endothelial wall of capillary blood-vessel.

c. A liver tubule.

d. Lumen of a liver tubule cut transversely.

Fig. 10. Portion of an osmic acid preparation of the liver of the Rat. (× about 400 diam.)

a. Spaces occupied by the network of blood capillaries cut longitudinally.

b. Similar spaces cut transversely.

c. Transverse section of a bile capillary lying between two liver cells.

d. A similar bile capillary lying at the angle where three liver cells meet.

Fig. 11. A similar preparation of the liver of the Mole. (× about 400 diam.)

a. Spaces for blood capillaries.

b. Section of a bile capillary lying between four cells. A similar one is seen near it, lying between three cells.

Fig. 12. Portion of a section of a cochineal stained specimen of the liver of the Pig, from the peripheral part of a lobule. (× about 400 diam.)

a. Nucleated wall of a capillary blood-vessel.

b. Liver cells showing a collection of granules where two or three cells are contiguous.

ON NERVE-TRACTS DEGENERATING SECONDARILY TO LESIONS OF THE CORTEX CEREBRI. By C. S. SHERRINGTON, M.A., M.B. (Preliminary.)

The object in view was to ascertain to what extent there is in the pyramidal tracts a grouping of nerve-fibres corresponding to the grouping of nerve-cells in the "cord-area[1]" of the cerebral cortex.

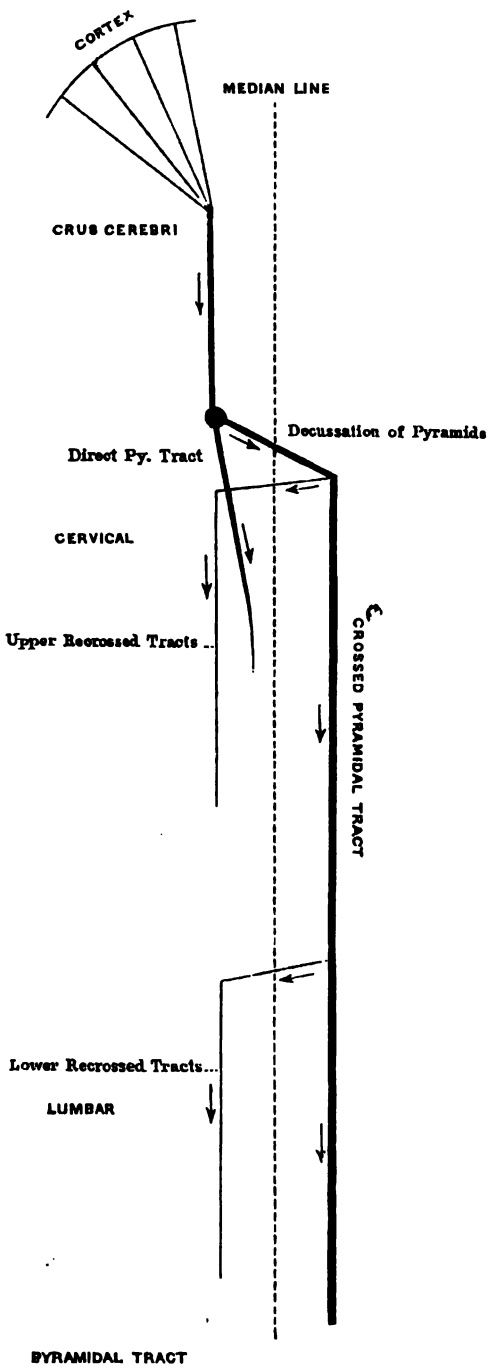
For this purpose a number of shallow lesions were made in the cerebra of monkeys (Macacus) and dogs. As the observations were to be chiefly on monkeys, a delimitation of the entire pyramidal tract in that animal was attempted at the outset. The tract was found to occupy as much as three-fourths of the transverse area of the crusta a little above the pons: it was also found to extend even as low as the origin of the coccygeal nerve-roots: thus, to be considerably more extensive than any existing account of it would seem to indicate. A peculiarity, hitherto undescribed, was also noticed in the tract in Macacus. In the cord of Macacus from the level of the 2nd cervical to the level of the 2nd lumbar nerve-roots a portion of the tract (one-fifth of the entire tract at the level of the 3rd cervical root) lies outside the direct cerebellar tract separating that latter from the periphery of the lateral column. That is to say, the direct cerebellar tract ascends not along the edge of the lateral column but within the crossed pyramidal tract, dividing that latter into a deeper lying main portion, and a smaller peripheral sheet.

With regard to the supposition that in a transverse section of the tract, e.g. in the upper cervical region, the fibres descending from the arm-area of the cortex might be found grouped more or less together in one portion of the tract, and on the other hand fibres from the leg-centre grouped more or less together in another portion of the tract, that supposition was not confirmed.

After a cortical lesion of even less than thirty square millim-extent the degeneration was found scattered over the whole transverse area of the tract. And this was true not only for the degeneration in the cord, but also for the degeneration in the crusta at the base of the brain.

The only evidence of a localized grouping of the fibres from the cortex to the cord, lay in the fact that the more mesial and anterior

[1] By the "cord-area" of the cortex is meant that area of the cortex injuries to which are followed by degenerations of nerve-fibres in the spinal cord. Cf. Sherrington, This *Journal*, Vol. VI. p. 177.



lesions in the so-called motor cortex were followed by degenerations which in the cervical cord were especially severe in those portions of the pyramidal tract abutting upon the direct cerebellar tract. Whereas after lesions in the more lateral and more posterior districts of the motor cortex those portions of the pyramidal tract which abut upon the cerebellar tract suffered less.

Enquiry was then made into the longitudinal localization of fibres in the tract.

It was found that after a lesion in the leg-area, encroaching little if at all upon the cortex of the arm-area, the degeneration in the cord stopped short in great part in the cervical region, especially in the cervical enlargement.

Again, after lesion in the arm-area, encroaching little if at all upon the cortex of the leg-area, the degeneration in the cord extended down through the dorsal into the lumbar, and even throughout the sacral region of the cord.

Following every cerebral lesion that gave a cord degeneration at all, and also following hemisection of the cord in the lower dorsal region, the resulting degeneration was found to be bilateral, i.e. the unilateral lesion caused de-

generation in the pyramidal tracts below in both lateral columns of the cord. The amount of degeneration on the recrossed side varied extremely, from less than one-hundredth to more than one-sixth if expressed in terms of number of degenerated fibres in the opposite crossed pyramidal tract.

From the degenerations the following points were made out for the "recrossed tracts[1]":—

i. They consist of fibres derived from the crossed tract of the opposite half of the cord, and are connected directly, i.e. without the intermediation of nerve-cells, with that cortex which is the place of origin of the crossed tract whence they come.

ii. Their path of connection with the cortex twice crosses the median line, the proximal crossing being at the pyramidal decussation.

iii. They are largest in the cervical and lumbar enlargements of the cord.

iv. They occupy the same area in a transverse section of the cord as does the crossed pyramidal tract, amongst the fibres of the crossed tract from the left hemisphere being scattered those of the recrossed tracts from the right hemisphere.

v. Following small cerebral lesions in certain portions of the cortex degeneration of the recrossed tracts in the cervical and lumbar enlargements may exist, and for a considerable length of the intervening dorsal region no degeneration be present on the recrossed side.

vi. Division of one lateral column in the dorsal region (lower) causes below a degeneration on the side opposite to the lesion as well as on the same side as the lesion—i.e. a degeneration of the recrossed lumbar tract as well as of the main crossed tract.

By counting degenerated fibres in the tracts it was found that the fibres not only of the recrossed but of the crossed tracts do not diminish in number from above downward in a regular manner. After lesions of leg-area and arm-area they diminish in number most rapidly in the lower portions of the lumbar and cervical enlargements respectively: after face-area lesions in the portions of the pyramid distal to the pons.

As traced from above downward they may actually not diminish in number but increase. Especially is this true of the upper portions of the cervical and lumbar enlargements. The fibres must therefore branch as they pass downwards. This branching throws an obvious light on the nature of the recrossed tracts.

[1] This nomenclature was proposed in a paper in this *Journal*, Vol. VI. 177, for reasons there explained. The arguments there adduced are supported by the present results.

With it may also be connected a frequency of arrangement of the degenerating fibres in a scattered degeneration in pairs. The fibres thus found in pairs may to avoid periphrasis be termed "geminal" fibres.

"Geminal fibres."

i. Each of the two fibres is a counterpart of the other in size and in the stage of degeneration which it has reached.

ii. The fibres are almost always quite large or large-medium; geminal fibres of quite small size I have not found.

iii. Occasionally to be seen are "geminal" axis-cylinders, enclosed in a medullary sheath common to both.

iv. Geminal fibres are relatively as well as absolutely very much less frequent in the recrossed than in the crossed tracts, although the recrossed tracts contain a far larger percentage of large and large-medium fibres than do the crossed tracts.

It is suggested that the "geminal" fibres are derived from parent fibres by dichotomous division. A fibre of the pyramidal tract may give rise to more than one branch; or its branches may themselves again divide.

It was further shown that, although the belief that the motor area of the cortex and the "cord area" of the cortex are conterminous rests upon no very accurate observations, still the two areas are so in certain directions very nearly indeed, if not absolutely.

Considerations suggest that the pyramidal tract is a path of cortical visceral (splanchnic) as well as of cortical somatic fibres—hence fibres descending from the arm-area of the cortex into the lumbar region of the cord. Insistence may be laid upon the pyramidal tract as a line of *direct* communication of each hemisphere of the brain with *both* halves of the spinal cord, not with one only—as generally described.

Other observations showed that

i. The fibres of the fornix degenerate from behind forwards.

ii. After lesion in the "cord-area" of the cortex fibres degenerate which pass through the corpus callosum into the opposite hemisphere.

iii. Degenerations in the corpus callosum are entailed also by lesions situate in the cortex posterior to the cord-area, as also by lesions lateral to the cord-area, and anterior to the cord-area.

iv. The interhemispheral degenerations tend to scatter and do not pass chiefly between "identical" areas of the two hemispheres.

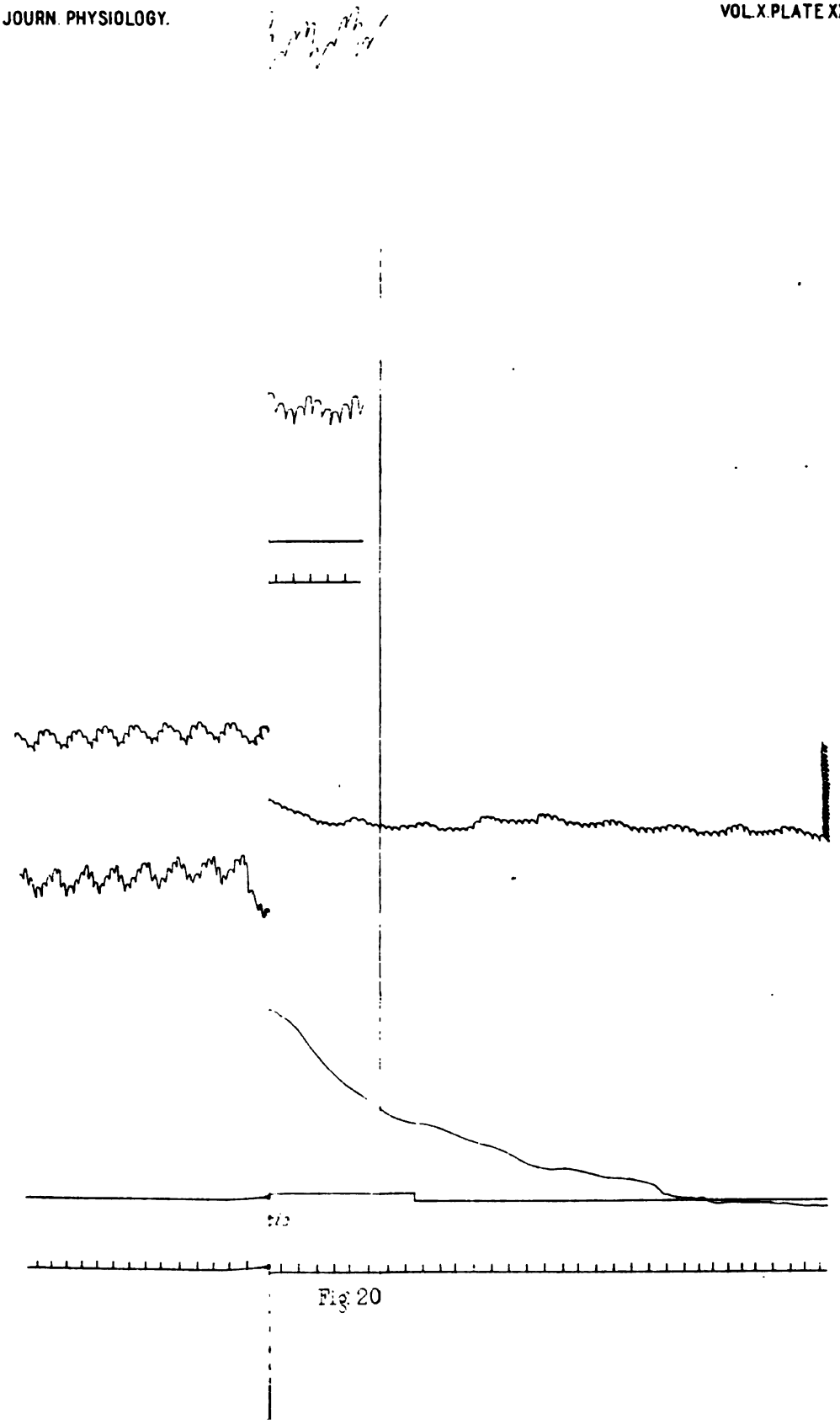


Fig. 20

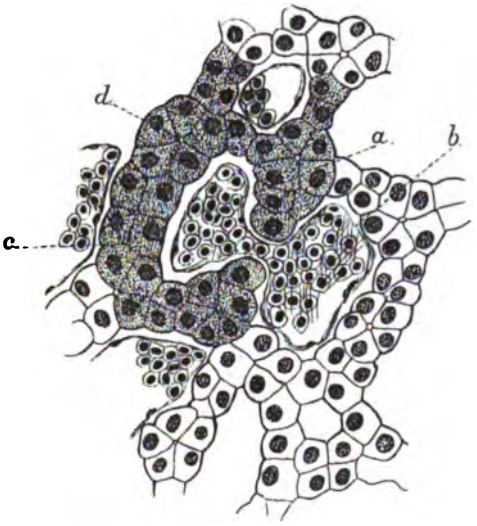


Fig. 9.

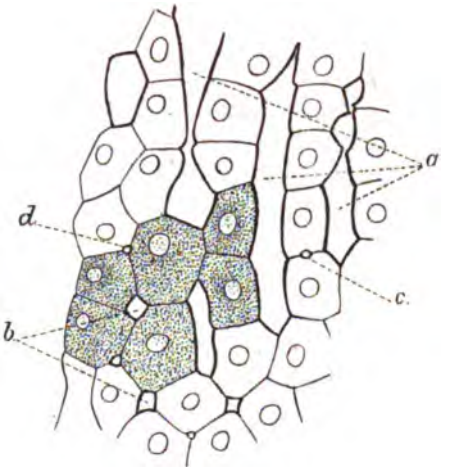


Fig. 10

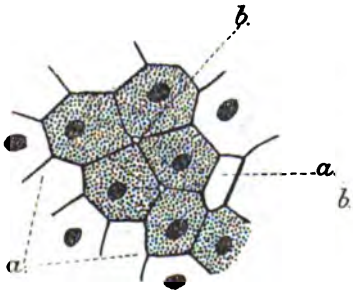


Fig. 11.

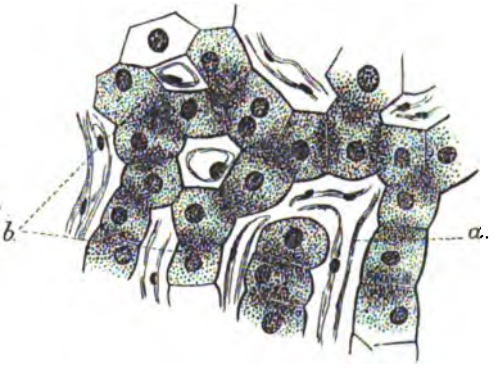


Fig. 12.

ON THE HISTOLOGY OF THE MUCOUS SALIVARY GLANDS, AND ON THE BEHAVIOUR OF THEIR MUCOUS CONSTITUENTS.

By J. N. LANGLEY, M.A., F.R.S., *Fellow of Trinity College, Cambridge.* Plate XXX.

A GOOD deal that is given in this Paper in some detail I have already briefly described in the *Proceedings of the Royal Society*, Vol. XL. p. 362, 1886.

Some of the conclusions which I then drew I have since seen reason to change and to reconsider. And in especial, the view that I gave as to the structure of the mucous cell requires reconsideration. For whilst I have obtained further evidence of the existence of separate particles of mucin, the granules, both in the fresh and in the hardened mucous cell, and of the existence of a network in the cells after treatment with various reagents; the evidence for the existence in the living cell either of a network or of mucin in any other form than granules is much weakened by some facts with regard to the behaviour of the gland which I had not observed at the time of my earlier account.

The discussion of the structure of mucous cells will best be taken later, after a description of the appearance presented by the cells in various conditions.

The matters I propose to deal with are, the microscopical appearance of the fresh and of the hardened gland, the various bodies that can be seen in saliva, the changes which take place in the gland cells during secretion, the nature of the demilune cells, the nature of the mucous substance in the gland and in saliva.

In the following account I describe, unless otherwise mentioned, the orbital and the sub-maxillary glands of the dog. I have compared with these a certain number of other mucous glands, viz. in the dog, the pharyngeal mucous glands; in the cat, the orbital and sub-maxillary glands; in the mole, the sub-maxillary gland; and in the rat and guinea-pig, the mucous portion of the sub-maxillary gland. In all these, with the exception of the mucous portion of the sub-maxillary gland of the

rat, the mucous cells differ, so far as I have observed, chiefly in the size of the mucous granules and in the amount of proteid substance which they contain. The sub-lingual gland, since it has some special features, I shall describe separately.

Part I. Chiefly on the Microscopic appearances of the Fresh Gland, and the effect of various reagents on these appearances.

Appearance of the fresh gland. It is not easy to obtain a thin fragment of a fresh mucous gland. A piece fairly thin at the edges may be obtained, either by preparing a section free-hand with a razor, or with the aid of a Valentine's knife, or, in the case of softer glands, by cutting off a small piece with a sharp pair of scissors.

Thin sections can of course be obtained from a piece of frozen gland, but unfortunately freezing alters considerably the appearance of the cells; after freezing the granules of the mucous cells are not distinctly distinguishable from the rest of the cell substance, nor do they become so on adding salt solutions (cf. p. 436), the cell outlines are distinct, the nuclei often visible, the granules of the demilune-cells and of the ducts are faintly seen. If the piece of gland be soaked in NaCl 5 p.c. before being frozen a few of the mucous granules may be preserved here and there in a section.

When a thin section or fragment of a quite fresh gland is examined without the addition of fluid, all the alveoli have a ground-glass or an irregularly granular appearance, but distinct separate granules are usually not seen; there is, in the thicker parts of the preparation, little or no difference between albuminous and mucous alveoli[1], or between demilunes and mucous cells. But in parts of the preparation consisting of a single layer only of cells, all the cells are seen to contain feebly refractive granules, spherical or nearly so, those in the mucous cells being larger and more distinct than those in the albuminous and demilune cells. Occasionally the mucous granules are distinct in the

[1] It is a generally accepted fact that the salivary glands are branched tubular glands. This has been insisted on recently by Flemming; in the absence, however, of any convenient term, I shall keep to the custom of calling 'alveoli' the oblique and transverse sections of the secreting terminal tubes as they are seen with the microscope. Such 'alveoli' may consist either of albuminous or of mucous cells, or of mucous cells with a few albuminous cells lying apparently outside them. In the latter case the albuminous cells are usually called demilune cells. I shall for the present follow this usage, although I do not think that there is any difference, except in shape, between demilune cells and the cells of albuminous alveoli.

freshly mounted specimen, possibly in consequence of a little more lymph than usual having soaked into it. In the resting state of the mucous cells, the granules stretch throughout them. In young animals, and in animals killed during digestion, there may be a narrow outer margin which is very faintly granular, less distinctly so indeed than the demi-lune cells. The margin consists of a small amount of proteid substance lying at the base of the cell. The nuclei of the cells are not visible, and it is often barely possible to make out the cell-outlines; in some cases, however, and not infrequently in the glands of the cat, the limits of the cells as transparent lines can be fairly well seen, apparently because of the comparative thickness of the limiting layer of the cell.

After the specimen has been mounted for some time, the granules, and especially those in the mucous cells, become more distinct. The granules are closely packed and have no definite arrangement. In the mucous cells there are ten to twelve in a line, stretching from the lumen to the basement membrane. Since the granules do not vary much in diameter in different cells, the number of granules in any one cell depends chiefly upon its size. As a rule the number of granules in a cell may be taken as varying from 125 to 250.

In the process of mounting the specimen some of the cells may have been more or less broken up; in such case, if there is a little fluid clinging to the specimen, the contents of the cells mix with it. The granules are then distinctly seen, although their outlines are not so sharp as those of the granules in a similar preparation of the parotid gland; still some care is required to distinguish them from the small fat globules which are also usually present.

The granules are surrounded by a clear homogeneous substance which causes them to be arranged in rows. This is better seen when the cover-slip is gently pressed, so that the granules can be watched as they slowly stream out of the alveoli.

Although, as I have said, the granules do not vary appreciably in size in different cells, yet they vary in size in any one cell; their average size is somewhat greater than that of the proteid granules of the parotid gland cells. Most of the mucous granules of the sub-maxillary gland of the dog have a diameter of $1{\cdot}0\mu$ to $1{\cdot}5\mu$, but a considerable number of smaller ones and a few larger ones are always present; in the orbital and in the pharyngeal mucous glands of the dog the granules are a little larger, most being $1{\cdot}25\mu$ to $1{\cdot}75\mu$ in diameter.

Some of the alveoli usually undergo changes different from that

described above. In some, the granules become less and less distinct, and the substance between them becomes more and more distinct, so that the cells appear at first sight to contain a network with small meshes, viz. 8 to 12 lumen to basement membrane (Fig. 2, Pl. XXX.). On closer examination the apparent network can here and there be seen to be simply the optical section of the small amount of substance existing between the granules, but in most cases it is not sufficiently distinct to justify any conclusion with regard to it.

After an experiment in which the outflow of saliva from the duct has been retarded,—e.g. when the secretory pressure is observed—the cells often look vacuolated. The apparent vacuoles consist of swollen mucous substance, they are not all spherical; here and there such a swollen mass may occupy nearly the whole of the cell in which it lies. Apart from cases in which the outflow of saliva from the gland has been obstructed, a vacuolated appearance is rare. When it occurs, the mucous granules may still be rendered obvious by irrigating with sodium chloride 1 p.c. or stronger.

The ducts of the lobules in the fresh gland have a slightly yellowish tint, this is much more marked in the sub-maxillary than in the orbital gland. The outer and larger portion of the ducts, occasionally almost homogeneous, is usually finely and indistinctly granular (cf. Fig. 8 (*a*), Pl. XXX.). The striation which is so obvious after most methods of hardening is not seen in the fresh gland; but it usually becomes more or less apparent when the specimen is kept. The granules for a time become more obvious, then they swell, and as they do so, become paler, arranged in rows, and often elongated, at the same time the cell-substance between them becomes more distinct; owing to these two changes the ducts acquire a striated appearance (cf. Fig. 8 (*b*)). Occasionally the duct cells, besides containing small fat globules chiefly in the neighbourhood of the nucleus, contain also large fat globules about the size of the nucleus; these are not uncommon in the submaxillary gland of the cat.

Effect of sodium chloride solutions. On irrigating a freshly mounted specimen with sodium chloride ·6 p.c., the granules of the cells become fairly distinct. The mucous granules which have been set free by the breaking up of some of the cells, swell and become less refractive, the outermost lying granules, which have very little viscid mucous substance surrounding them, become difficult to make out.

Some of the granules suddenly disappear, bursting like bubbles. On this point it is necessary to say a word or two, although it anticipates

a part of what must be described later. The manner in which the granules disappear with a number of reagents is like that in which granules of the œsophageal glands of the frog and of the gastric glands of many vertebrates disappear on adding hydrochloric acid. In these cases we speak without hesitation of the granules being dissolved. In the case of mucin granules the matter is different. It is true that alkalies which dissolve mucin—as far as it is dissolved in any reagent—cause them to 'burst,' but on the other hand, a similar bursting of many granules can be seen on irrigation with a saturated aqueous solution of mercuric chloride, in which mucin of saliva forms at most a ropy clump. I take it, that the granules on bursting may form either a viscid solution or a ropy mass. Under the microscope, the granules disappear as such completely; they form a continuous, transparent, more or less viscid mass. Microscopic examination affords no satisfactory means of determining in such a mass how far the mucin is dissolved and how far simply swollen up. The disappearance of the granules in the above-mentioned manner I shall then simply speak of as the bursting of the granules.

When a piece of gland is teased out in NaCl 1 to 5 p.c., the fragments become less transparent and whiter; under the microscope the alveoli are much more opaque than in the fresh gland, and are packed with granules, nuclei are not seen in the alveoli, and cell outlines rarely. The mucous granules have very sharp outlines, whether they are seen in the alveoli or lying free in the fluid. Occasionally in 5 p.c. NaCl the granules stick together in rows or in clumps as they are pressed against one another, but they do not coalesce.

On irrigating a slightly teased fragment of a fresh gland with NaCl 5 p.c., it is seen that the contents of the alveoli as they stream out into the fluid, form first a clear bleb, closely beset with granules (cf. Fig. 1); then the bleb swells and the granules become arranged in lines parallel to the surface of the bleb.

The clear substance is swollen mucin; for on irrigating the specimen with strong acetic acid or alcohol, the bleb shrinks and becomes changed into a membranous looking mass of mucin, such as is produced by irrigating a small drop of viscid saliva.

In the specimen irrigated with sodium chloride solution, the bleb continues to swell, but keeps for a time a fairly sharp outline towards the fluid; later this disappears and the granules appear to lie free in the fluid. With NaCl ·6 p.c. the clear mucous substance swells more rapidly, so that there is for a short time only, or not at all, a line to be

seen marking the limit of the cell contents; in consequence the granules are usually arranged in lines parallel to the direction of flow. With NaCl 1 p.c. a few granules may burst. By pressing the cover slip it will be seen that the granules near the alveoli are still surrounded by a viscous substance.

On continuing the irrigation with 5 p.c. NaCl, the groups and rows of granules are gradually washed away from the edge of the specimen. The rows of granules as they are carried away by the current often turn over bodily, the constituent granules not being separated from one another. But here and there granules are separated from a row, so that in the further course of the current no row consists of more than two granules; these separate when the viscous substance around them becomes diluted to a limpid fluid. A delicate connecting filament can sometimes be observed between two granules (cf. Fig. 4 (*b*)) as they are being rolled over. Since no such connecting filament between the granules can be seen inside the cells, it probably indicates that the granules here and there stick together where they come in contact.

In 10 p.c. sodium chloride, the appearance of the granules is much the same as in 5 p.c.; but a smaller bleb is formed where the cell contents are poured out, and this swells much more slowly, and the rest of the cell substance is more transparent. In 20 p.c. and in stronger solutions of sodium chloride, sometimes also in weaker, the granules are apt to be elongated or irregular in shape instead of being spherical (cf. Fig. 4 (*c*)); they are more apt also to cling together in small groups; in some of these groups the separate granules may be barely distinguishable. On irrigating with 5 p.c. NaCl after 20 p.c. the bleb of mucin, seen where the contents of the cells have streamed out into the salt solution, swells, and on irrigating with 1 p.c. NaCl the bleb of mucin swells still more and some of the granules burst.

I have said that when the more dilute salt solutions are used, some of the granules as they stream out of the cells may be seen to burst; with 5 p.c. and stronger solution of salt I have never been able to see a granule burst except when the gland has been kept for some time.

In the salt solutions the mucous granules are preserved for a considerable period. Gradually, however, they swell slightly and their outlines become faint, finally they disappear. The rate at which the alteration proceeds depends in part upon the strength of the salt solution, being slower with a 5 p.c. than with a 1 p.c. solution, and in

part upon the absence of viscid, swollen or dissolved mucin around them. Thus, when a piece of gland is finely chopped up, and shaken with 5 p.c. salt solution, a more or less viscid fluid is obtained, and there is a ropy mass around the fragments of the gland; at first granules are fairly plentiful in the fluid, the ropy mass and the cells are thickly beset with them; in a day or earlier the granules may have disappeared from the fluid but still be found in the ropy mass; they largely disappear from the ropy mucin at a time when they are still fairly numerous in the cells; in these the granules may remain distinct for several days. Up to a certain stage many granules, not or barely seen when mounted in the fluid they have been kept in, may again be rendered obvious by adding a stronger salt solution.

Isolated mucous cells may now and then be obtained when a fragment of the gland is teased out in sodium chloride 5 p.c. mounted, and the cover-slip cautiously pressed. They are more common when a piece of the gland is finely chopped up with a scalpel and then well shaken in a small quantity of the salt solution. By either method they are more numerous if the gland is allowed to stay in the body for a day before being taken (cp. p. 451). Isolated cells may more frequently be obtained from the submaxillary and orbital glands of the cat than from the sub-maxillary gland of the dog: I have very rarely obtained them from the fresh orbital gland of the dog. I am inclined to connect the varying ease of isolation of the cells with the amount of proteid substance, relatively to mucous substance in them. A certain amount of proteid substance must be present in order to prevent the swelling of the mucous substance leading to a bursting of the cell; the easier isolation of the cells some time after death is similarly probably due to a coagulation of the protoplasm of the cells. A mucous cell isolated in 2 to 5 p.c. sodium chloride appears as a mass of highly refractive spherical granules held together by a barely visible cell-substance (cf. Fig. 5 (*a*)); it is more or less columnar, never pear-shaped or globular, as it is after isolation in chloral hydrate; it does not show a cell-process; the nucleus lies close to the basal end of the cell.

When a gland is allowed to remain in the body more than a day after death before being teased out in salt-solution the isolated cells often contain less than their original number of granules. The rate of disappearance of the granules varies a good deal. In the glands of the dog I have generally found the granules numerous for two days after death; but in one or two observations on the orbital gland of the cat, whilst the cells isolated, immediately after death, in 5 p.c. NaCl were

densely granular throughout, the cells isolated two days after death varied widely in granularity, some of them containing three or four granules only, scattered in the transparent mass of the cell; these cells though less angular than those in which the granules were preserved were not much swollen, but their outline was doubly contoured, differing thus from the cells in which the granules were for the most part unaltered (cf. Fig. 6 (*e*)).

The *nuclei* in sodium chloride ·6 to 5 p.c. are the less obvious the fresher the specimen. They are more distinct in the demilune and duct cells than in the mucous cells. They are all spherical, very transparent, and may show one or more nucleoli; this is constantly the case when the gland has been left in the body some time before being examined. Solutions of sodium chloride containing more than 2 p.c. of the salt cause the nuclei to swell up; the extent of this swelling varies considerably in different glands.

Demilune cells isolated in salt solution are more or less distinctly granular, the granules being one-half to one-third the size of those in the mucous cells (Fig. 5 (*c*)). The cells are usually in groups of two or more, the cell-outlines are very indistinct and often not visible, they may however be brought out by adding acetic acid and ferrocyanide of potassium and by various other reagents. Bi- and multi-nuclear demilune cells I have not seen. The demilune cells in the alveoli are often made by salt solutions more refractive and nearly homogeneous in appearance.

The *duct cells* become in 5 p.c. sodium chloride more distinctly granular, the granules extending to within a short distance of the free border of the cells.

The sub-maxillary gland of the cat differs slightly from the mucous glands of the dog. It does not as a rule show a bleb of mucin, as the contents of the alveoli pour out into the salt solution; this is no doubt in part due to the amount of mucous relatively to proteid matter being less in the gland cells of the cat than in those of the dog; it may also be in part due to a slight difference in the character of the mucin; such that in the sub-maxillary gland of the cat, it is less apt to form a ropy mass. A piece of the sub-maxillary gland of the cat mounted in salt solution shows a distinction between demilunes and mucous cells less clearly than the mucous glands of the dog in like condition.

Effect of sodium carbonate. Most of what has been said above as to the effect of sodium chloride holds also for sodium carbonate. In sodium carbonate solutions, however, the swollen mucous substance has

a larger volume than it has in solutions of sodium chloride. So when a piece of gland is chopped up in sodium carbonate solution, the ropy mass obtained is of greater bulk than when sodium chloride is used.

If a small piece of fresh gland is irrigated with at 1 to 3 p.c. sodium carbonate some of the cells burst and their contents are poured into the surrounding fluid. The clear swollen mucous substance can then be faintly seen in the neighbourhood of the alveoli; as it spreads out into the fluid it can no longer be seen, although the granules remain obvious.

In sodium carbonate solutions ·5 to 2 p.c. the granules swell up more than they do in sodium chloride solutions of the same strength. The granules remain unaltered for a considerable time; thus when a piece of gland is chopped up in so dilute a solution as ·2 p.c. Na_2CO_3, provided not too much fluid is taken, the cells may show granules for a day or even for two days; the greater the quantity of fluid and the more finely the piece of gland is chopped up, the more quickly the granules disappear.

Other salts of the alkalies and salts of the alkaline earths in general affect the granules, as do sodium chloride and carbonate.

Nature of the granules. As the granules form much the greater part of the mucous cells it is clear they must be mucous in nature. And this may be shown in the following amongst other ways. A small piece of gland is hardened in absolute alcohol, and a section prepared free hand, the razor being moistened with strong alcohol; the section when mounted in strong alcohol shows dark shrunken granules filling up the cells and forming the conspicuous feature of the section. In successful preparations the number of granules from lumen to basement membrane is 9 to 12, as in the fresh gland. On slowly irrigating the specimen with alcohol about 70 p.c. the granules swell up and become more spherical, resembling then closely the granules seen in a piece of fresh gland mounted in 2 to 5 p.c. salt solution. On irrigating with alcohol a little more dilute, the granules suddenly swell and form the transparent mass typical of mucous cells which have been hardened in alcohol and mounted in glycerine. At the same time the cell-outlines and a wide-meshed cell-network become distinct. The change is more rapid and greater if water instead of dilute alcohol is added. On replacing the dilute with absolute alcohol the cells return more or less to their original appearance. There is, however, great variation in this respect, the variation depending on the extent to which the granules on swelling up stick together. And this is probably the explanation of

the absence of granules in glands hardened in absolute alcohol, stained with aqueous or dilute alcoholic solutions and mounted in Canada balsam, in the manner adopted by Heidenhain and by Lavdowsky. At any rate, as I shall show in speaking more in detail of the hardened glands, the mucous granules can be preserved and deeply stained in sections of a gland hardened in absolute alcohol; and remain distinct on mounting the section in Canada balsam.

It follows from what has been said, that when a piece of gland is treated with not too dilute salt solution, part of the mucin of the gland either dissolves or forms a continuous ropy mass, and part remains as mucin granules.

The mucin which swells up might arise (*a*) from a more soluble form of mucin existing around the granules, (*b*) from a solution of some of the granules. In the latter case the cause of the solution might be either that some of the granules are more soluble than the rest, or that on adding salt solution the fluid first taken up by the cell is sufficiently diluted by lymph to cause a solution of a few of the granules. The latter is unlikely, since in serum the granules are only slowly dissolved (cf. p. 451), but the discussion of these points must be deferred until we have considered the appearance presented by the hardened gland.

General points of behaviour of Mucous Cells with reagents.

In order to observe the effect of reagents upon the various constituents of the gland cells, I have either irrigated with the reagent a specimen teased without fluid or teased in ·6 to 5 p.c. sodium chloride solution, or I have teased a small piece of the gland direct in the reagent.

Before giving a detailed account of the action of reagents, other than those already given, upon the various constituents of the cells of mucous glands it will be convenient to mention some commonly recurring points in the behaviour of the mucous cells. When a piece of mucous gland is teased out in salt solution 1 to 5 p.c., so that isolated cells and granules are obtained, and the specimen is irrigated with water, alkalies, mineral acids, acetic acid, or with a number of other reagents, the mucous granules disappear and a network comes out more or less distinctly in the cells. The more isolated granules burst like bubbles (cf. p. 437); when one of the row of granules thus bursts, the others are forcibly thrust asunder; on slowly irrigating with not too strong solutions, the granules first swell up and become pale; on

irrigating more rapidly the granules swell suddenly, bursting almost immediately afterwards, or they may burst without any previous swelling being visible. Where the granules are closely packed together, as in the cells and in the mass of broken-down cells streaming out of the gland, some only of the granules, except on irrigation with alkalies, are seen to burst; the others swell, become pale and are lost to view, —apparently as they swell they coalesce with one another or with already swollen mucin.

The granules, even when they burst, never give rise to a vacuole in the cell. When they have disappeared they cannot be rendered obvious again by irrigating with strong salt solution.

An isolated mucous cell on irrigation with one of the above-mentioned reagents swells up, becoming pear-shaped or globular. The swelling of the cell is caused in the main if not entirely by the swelling of its granules; these run together, forming a continuous mass except for the proteid network in it. With some other reagents, for instance with 60 p.c. alcohol, the fusion of the granules is less complete. On treatment with reagents, such as absolute alcohol, which causes mucin to shrink, the cell may then shrink as a whole, or it may shrink with some of the granules or clumps of granules in it, shrinking separately and so forming spaces in the cell. As the granules disappear from the cells a network comes into view, and the cell-outlines become sharply contoured. In the isolated cells the network has wide meshes, the number of meshes being as a rule 4 to 6 in the length of the cell (Fig. 5 (*b*)); the meshes are not quite equal in size, and the fineness of the fibres and the size of the nodal points varies slightly with the strength and nature of the reagent. In cells which are not isolated, the meshes of the network are generally smaller and less distinct, there may be 9 to 12 in the length of the cell.

Fig. 5 (*b*) shows the changes produced in the isolated mucous cells, Fig. 5 (*a*) by the addition of 1 p.c. hydrochloric acid. A similar change is produced by many other reagents; we may mention here acetic acid to which a little ferrocyanide of potassium has been added as showing that the network does not contain any mucous substance and is in consequence different from the network described by Schiefferdecker and by List in the analogous cells of certain mucous membranes. A wide-meshed network in mucous cells was first described by Lavdowsky, a fine-meshed network in them was first described by Klein. I shall consider their relations more fully later in dealing with the appearances shown by the cells after hardening.

The outline of the cells on irrigation with dilute acids, is in optical section more or less obviously beaded (Fig. 5 (*b*) and Fig. 7 (*a*)); there are usually twelve to fifteen small swellings in the length of the cell, in some cases the outline of the cell appears to be discontinuous in optical section, the limiting layer of the cell looking as if it were a perforated membrane. Similar small swellings have been mentioned by Schiefferdecker as occurring in cells treated with Müller's fluid. Now and then, and especially after treatment with chloral hydrate, the beading can be followed into a small-meshed network in the limiting layer of the cell (Fig. 7 (*b*)). The nature of this I shall consider later.

Alkalies. When a teased-out specimen of a fresh gland, either with or without the addition of sodium chloride, is irrigated with dilute ammonium, sodium or potassium hydrate, the isolated granules instantaneously disappear like bubbles bursting; soon they disappear in the same manner from the cells, though without any formation of vacuoles and the whole specimen becomes transparent, the fat globules stand out conspicuously. A piece of gland teased out in one of these fluids is, as is well known, changed into a ropy mass.

Water, when added slowly to a specimen teased out in salt solution, causes the granules to swell up considerably. The outlines of some of the granules becomes fainter and fainter, until they are no longer seen. The swollen pale granules can for a time be again rendered very distinct by adding 5 p.c. salt solution.

On rapidly irrigating a teased-out specimen the granules swell up or burst as they do with alkalies.

In my preliminary account (*Proc. Royal Soc.* Vol. 40, p. 362, 1886) I said that after irrigating a piece of gland with water or with dilute mineral acids—pale, very slightly refractive masses could be seen, consisting apparently of swollen granules. The persistence of the granules in this case was, I find, due to the slow diffusion of the fluid through the mucous mass surrounding them.

As the mucous granules disappear, small pale irregularly shaped granules and granular clumps become visible. These are the broken-up fragments of the proteid portion of the cells.

The isolated mucous cells on irrigation with water swell up, the granules in them become pale and indistinct, and may occasionally be seen to burst; the cell-outline becomes fairly sharp and a wide-meshed network becomes more or less obvious. The cell-outline and network is, however, much less distinct than after addition of acids.

A piece of gland teased out in water becomes a jelly-like clump,

less readily the more proteid substance there is in the cells. When the clump is examined under the microscope, the cells are swollen, without distinct network as a rule, and with moderately distinct cell outlines. No mucous granules are seen; in the transparent jelly-mass are pale, irregularly granular particles, resulting from the breaking-up of the limiting layer of the cell and of the cell-network.

The granules of the duct cells become larger and much paler, so that the ducts often look densely vacuolated, the cell-substance between the apparent vacuoles becomes very distinct (Fig. 8 (*c*)).

In water the nuclei of the mucous cells may be either disc-shaped, homogeneous and refractive, or spherical, pale, with one or more nucleoli; in the latter case they are made more obvious by irrigating with an aqueous solution of congo-red.

Hydrochloric acid. When a specimen teased out in salt solution is irrigated with hydrochloric acid ·2 to 1 p.c. the effect is much like that of irrigating with water. The chief differences are that the limiting layer of the cell, the cell network and scattered proteid particles are more distinct, and that a membranous looking precipitate of mucin takes place. On further irrigation or with stronger acid the membranous precipitate again swells up. On irrigating with HCl ·01 p.c. all the cells, and especially those of the ducts, become cloudy.

A piece of gland teased-out in hydrochloric acid does not form a swollen jelly-mass as it does in water, but on examining it under the microscope it is seen that many of the cells have burst and that their contents form a membranous mass on the outside of the specimen.

The scattered proteid particles spoken of above stain deeply with methylene blue and with most other colouring matters. A not inconsiderable number of them are spherical; in isolated cells—especially of the orbital gland of the dog treated with hydrochloric acid—similar small proteid granules are seen attached to or forming part of the network (Figs. 6 (*d*), 7 (*a*)). The nuclei of the mucous cells, spherical in 5 p.c. salt solution, shrink on adding dilute hydrochloric acid. Hydrochloric acid and nearly all reagents (except alkalies) which cause the mucous cells to swell, make the demilunes very distinct; they become irregularly granular.

Notwithstanding the very considerable swelling of mucin which takes place in the isolated cells on irrigation with HCl 1 p.c., it is remarkable how seldom a plug of mucin is seen to project from the free end of the cell. I have only seen this in the fresh gland, when a piece

has been irrigated first with dilute ammonia and then with hydrochloric acid (cf. Fig. 6 (*c*)).

Nitric acid. Irrigation with nitric acid causes the granules to disappear from a teased out fragment much less quickly than does irrigation with hydrochloric acid. The granules protected by the membranous mass of mucin swell up considerably, but keep for a time a fairly distinct outline. Even when a piece of gland is teased in 5 p.c. nitric acid some swollen granules may still be seen. With strong nitric acid, the cells become transparent and take a yellow tinge.

Osmic acid. On irrigation with osmic acid ·5 to 2 p.c. the granules swell up, it may be to twice their normal size, and become difficult to make out; if much osmic acid is present they gradually disappear, apparently being dissolved. If the specimen, after addition of osmic acid, has a small quantity of fluid only, and is kept in a moist place, the granules take a brownish tint and remain for a considerable time fairly obvious. By exposing a piece of freshly teased-out gland to the vapour of 1 to 2 p.c. osmic acid, the granules can be kept almost indefinitely. To do this a cover-slip is put on the specimen and well cemented at the edges.

In a previous paper I described three kinds of 'granules' as issuing in a freshly mounted specimen from the cells of the sub-maxillary gland of the cat. (*a*) Granules which swell up, lose their distinctness of outline, and then disappear: these are the mucous granules (*b*) others which with osmic acid become pale granular clumps; these are the proteid portions of the cells (*c*) still others which stain deeply with osmic acid: these are fatty globules, as mentioned by Lavdowsky, they are not infrequently found in mucous glands.

Cells or groups of cells which have been separated during teasing, become pale and swell up somewhat on irrigation with osmic acid, their outlines become very obvious and are usually beaded; in the cells an indistinct wide-meshed network becomes visible. The cell network with osmic acid as with other acids is much more distinct if the specimen before irrigation has been teased in 5 p.c. NaCl, than if it has been teased without the addition of fluid.

Osmic acid and saliva. In connection with the solution of the granules by osmic acid, I may mention the effect of osmic acid on saliva. When the sub-maxillary saliva of the dog is allowed to run into 1 to 2 vols. of 1 to 2 p.c. osmic acid, it forms at first a viscid or ropy mass; if the saliva is very thick it turns as it falls into the osmic acid into a firmish jelly. In a few hours, the time depending upon the

viscidity of the saliva, the ropy or jelly mass breaks up on shaking into flocks, and the fluid is limpid; later the flocks disappear, the limpid fluid takes a yellow-brown tinge, gradually turning to black.

Acetic acid and salt. When a piece of gland is mounted in 5 p.c. sodium chloride solution and irrigated with acetic acid of any strength the granules swell up and disappear, as they do with alkalies, water and mineral acids. The acetic acid causes the formation of a jelly-like mass of mucin, looking as it shrinks further, on further irrigation, more and more as a membrane. In this are seen small clumps of pale irregularly shaped granules. The latter are in the main proteid particles of the cells which have been partly dissolved, partly swollen up by the salt solution. It is possible that they may in part be mucin precipitated in a granular form. On irrigation with dilute acetic acid, as 1 p.c., there is an interval between the disappearance of the mucous granules and the appearance of the irregularly granular clumps spoken of above. The amount of the granular precipitate is roughly inversely as the amount of the membranous precipitate. In the sub-maxillary gland of the cat the membranous precipitate may be chiefly represented by filamentous fragments, whilst the granular precipitate is copious. In the orbital gland of the dog the granular precipitate is slight. The granular precipitate has sharper outline in 10 to 20 p.c. acetic acid than in the more dilute acid. The mucous cells on irrigation with acetic acid and salt swell up, and show a distinct network, the nuclei are made more distinct; they shrink with strong acetic acid, but little, or not at all, with weak acetic acid.

Solubility of mucin in acetic acid plus sodium chloride. The bursting of the granules in acetic acid requires a word of explanation. It is well known that the addition of sodium chloride to a solution of mucin delays the precipitation of mucin by acetic acid, so that a certain excess of acid can be added before precipitation occurs. This has been mentioned by Landwehr and by other observers. Mucin obtained from different sources behaves somewhat differently in this respect. Thus, according to Hammarsten[1], a solution of the foot-mucin of the snail in the presence of about $\frac{1}{4}$ volume of a saturated solution of sodium chloride is precipitated by acetic acid when it is very nearly neutralized, whilst the mantel-mucin of the snail (*op. cit.* p. 373) and the mucin of the sub-maxillary gland of the ox[2] can in similar conditions be made fairly strongly acid without a precipitate forming.

[1] Hammarsten, *Pflüger's Archiv*, Bd. 36, p. 424, 1885.
[2] Hammarsten, *Zeitschrift f. physiol. Chemie*, Bd. 12, p. 194, 1888.

I find that the mucin of the saliva of the sub-maxillary gland of the dog in the presence of a certain amount of sodium chloride is not precipitated by a large excess of acetic acid. Thus if to a few c.c. of saliva a little saturated solution of sodium chloride is added, the addition of an equal volume of melted glacial acetic does not precipitate the mucin, indeed it sometimes appears as if glacial acetic acid only precipitates the mucin when it also begins to precipitate the sodium chloride. Further, when a precipitate is produced in saliva by adding a little acetic acid, the granular and flocky part of the precipitate instantly disappears on adding a drop or two of strong salt solution, the jelly part of the precipitate swells up, becomes transparent, and more slowly dissolves. The addition of large excess of strong acetic acid is then without effect. Hence the bursting of the mucin granules of the gland by acetic acid in the presence of salt solution is not surprising.

Acetic acid. A greater or less number of mucous granules swell up and burst when a fragment of gland to which no salt solution has been added is irrigated with acetic acid. This is best seen in the orbital gland of the dog. A small piece is mounted and slightly pressed, and a portion of the edge watched where the cells have burst and poured out their contents; as soon as the acetic acid—1 p.c. to nearly concentrated—reaches this, a number of granules burst, others become pale, apparently running together, and the whole is turned into a membranous mass, having in it small irregularly granular fragments of the proteid portions of the cell.

Hence, then, with acetic acid the isolated granules and clumps of granules may either burst, i.e. in this case, swell up largely and run together to form a ropy mass[1], which is later precipitated as membranous mucin; or swelling somewhat at first may later shrink into irregular granules or irregular granular masses.

In the cells the granules swell up, run together and form a clear mass, in which a proteid network is not very distinctly seen. The primary swelling of the mucous granules with acetic acid is probably due to their containing a certain amount of salt or alkali; as this is washed out, the mucin shrinks.

On irrigating a specimen with dilute acetic acid, as 0·1 p.c., a number of granules usually take a more oily look and have a greater

[1] In my preliminary account I stated that the granules were shrunken on irrigating a teased out specimen with acetic acid or with alcohol. I had, at that time, only observed the effect of strong acetic acid and of 95 p.c. alcohol.

tendency to stick together. This also occurs in the more central parts of the specimen on irrigating with stronger acetic acid. The granules then resemble the spheres which are seen in viscid saliva on treatment with dilute acetic acid, but unlike these, they become pale on addition of stronger acetic acid. With acetic acid ·1 p.c. or weaker, all the cells become cloudy, the ducts more so than the alveolar cells.

When a piece of gland is teased-out in 5 to 20 p.c. acetic acid and mounted, a variable amount of mucin film is seen around the fragments of the gland; the gland cells have a ground-glass look, they barely show the cell-network and not very distinctly the cell-outlines and nuclei. If the specimen be irrigated with 5 p.c. salt solution, the membrane and the cells swell, the network and cell-outlines become distinct and the demilune cells become obvious, being then more refractive than the mucous cells. The addition of glacial acetic acid makes all the cells very transparent—the ducts being the first to become so—and beyond faint cell-outlines and sharply marked fat globules, little is seen; all the cells are considerably shrunken.

When a piece of gland is teased-out without addition of fluid, and then flooded with strong acetic acid, the membranous mass of mucin around the alveoli is crowded with faintly showing granules, spherical or irregular, these are chiefly the mucous granules fixed in situ.

Acetic acid and ferrocyanide of potassium. When a drop of ferrocyanide of potassium is added to a little, 5 to 20 p.c., acetic acid, and the mixture added to a teased-out fragment of gland, the isolated granules swell up and dissolve, the cells swell up, become transparent and show very distinctly the cell-outline and a wide meshed network.

Alcohol. Alcohol up to about 70 p.c. causes the isolated granules to swell up and disappear; in the cells the granules swell up and run together.

When a small piece of gland is placed in 30 to 50 p.c. alcohol it becomes surrounded by a ropy mass, but for a day a fragment of the gland teased-out in 5 p.c. salt solution may still show granules.

If a piece of gland be teased-out and then flooded with 80 p.c. absolute alcohol the isolated granules are shrunken, irregular, refractive; in the cells the granules shrink, often leaving spaces in the cells. When the granules in consequence of previous treatment, or in consequence of the slow penetration of the alcohol, have swollen up before shrinking, they may in shrinking remain connected by filaments, or they may shrink in groups.

Mercuric chloride. When a piece of gland is teased-out in 5 p.c.

sodium chloride and irrigated with a saturated aqueous solution of mercuric chloride, there is at first no change; later, as the sodium chloride becomes washed out, the isolated granules burst, a membranous precipitate is formed, the cells swell up, become transparent and show a network with a variable number of meshes. Much the same change is produced by 1 p.c. silver nitrate, 1 p.c. gold chloride; a saturated aqueous solution of picric acid causes the cells to swell more; chromic acid 1 p.c. causes them to swell less.

Chloral hydrate 2 *to* 5 *p.c.* Irrigated with this, the mucous granules swell up and become very pale; the cells swell and the granules in them cannot be distinguished. This fluid is commonly used for obtaining isolated cells; when a small piece of gland is placed in it the outermost cells burst, and their contents form a ropy mass. This remains for several days, but is gradually changed into an irregular granular deposit. Isolated cells are best obtained in about three days; the cells are then much swollen, most show with greater or less distinctness a wide-meshed network in their interior (Fig. 7 (*a*)); it may be made still more distinct by irrigating the specimen with ·1 p.c. osmic acid; sometimes the meshes near the nucleus are smaller and the bars of the network thicker than elsewhere. In some of the mucous cells—generally those in which the mucin is most swollen, and the inner network indistinct—there is a very marked fine-meshed network in the limiting layer of the cell (Fig. 7 (*b*)); the meshes vary somewhat in size; they are usually five or six sided. In such cells the limiting layer of the cell, apart from the network, is extremely faint; here and there fibres run from this network to the wide-meshed internal network. I use the phrase network in the limiting layer of the cell simply as descriptive of the appearances under the microscope, I shall later discuss whether it really is a network, or whether it consists of ridges on the limiting layer caused by the pressure exercised by the granules as they swell.

Speaking broadly the cells of the sub-maxillary gland of the dog swell less than those of the orbital gland, and the cells of the sub-maxillary gland of the cat less than those of the sub-maxillary gland of the dog. Within limits, the less the cells swell the less distinctly do they show a network.

Neutral ammonium chromate 5 *p.c.*, which is also often used for obtaining isolated cells, causes less swelling of the mucous substance than does chloral hydrate. When a piece of one of the more mucous glands, as the orbital of the dog, is placed in neutral ammonium

chromate, a small amount of ropy substance is at first found around the piece of gland, in a day or two this ropy mucin is converted into a flocky precipitate. The isolated cells show a network less distinctly than after treatment with chloral hydrate; the limiting layer is generally beaded. Some, especially from the sub-maxillary gland of the cat, may be isolated with the angular form the cells have normally in the gland.

Saliva. When a piece of gland is teased-out in saliva in which nothing is to be seen under the microscope, few or no granules are seen in the fluid but some are still seen in the cells. If thick viscid saliva be taken, the great majority of the granules are as distinct as if the gland had been teased in NaCl 1 p.c.; some however swell considerably and can scarcely be distinguished, except by addition of reagents, from the 'spheres,' which as we shall see later are normally present in thick saliva.

Blood serum causes a slight swelling of the granules only, but added to the fresh gland makes them much more distinct than they are normally. The nuclei are generally made fairly distinct as pale spherical bodies, with one or more nucleoli.

Staining of mucous granules. The staining power of the granules can of course only be tried with those staining agents which are soluble in dilute salt solutions. Methylene-blue in a 1 p.c. NaCl solution has a very variable effect; in one case only—that of the orbital gland of a dog a day after death—did the granules stain readily and deeply; usually some of the granules stain fairly and others very slightly, this may perhaps be due to the membranous precipitate of mucin which methylene-blue causes. Hæmatoxylin and the other reagents which I have tried do not stain the granules.

Post-mortem changes. If a gland be allowed to stay in the body for a day after death it becomes firmer, probably in consequence of a coagulation of its protoplasmic portions; when cut across, the exposed surface is more viscid than it is in a fresh gland. The mucous granules are unaltered except that they are rather more distinct in a piece of gland mounted without the addition of fluid. Such a gland has certain advantage for examination over a fresh gland, for since it is firmer, it is easier to get a thin section; moreover on teasing out a section in a saline solution some isolated cells are usually obtained, whilst on teasing out a fresh gland, fragments of cells and portions of alveoli are obtained, but—in the mucous glands of the dog,—rarely isolated cells. A gland may be kept three to four days, and still the mucous granules may be distinct on teasing out a fragment in salt solution; a very

gradual alteration, however, takes place in them, such that at first their outlines become indistinct, and later they are not to be seen as granules; moreover they may for a time be still obvious in strong salt solution. although they are not or are barely recognisable in dilute salt solution. The post-mortem change in the mucous granules consists then in a gradual increase in solubility, or in power of swelling up, in the salts of the alkalies.

When a gland has been kept for one day to two days in NaCl 5 p.c. the cell-network and the limiting layer of the cell are not brought out so distinctly by acids. The limiting layer is the first to become indistinct; in this stage the cells of the orbital gland not infrequently show a network between the peripheral meshes of which the clear mucous substance of the cell bulges (Fig. 6 (*d*)). Even without the addition of salt solution, the gland being left in the body, a similar alteration takes place; thus water, though bringing out fairly distinctly the network and limiting layer in cells from a fresh gland, may fail to do so if the gland be left in the body for a day after the animal has been killed, and proteid particles still present indicate a breaking up of the proteid part of the cells. When cells are isolated in sodium chloride ·6 p.c. from a gland which has been kept for a day and the specimen irrigated with water, it sometimes happens that the nuclei can be seen to be set free by the breaking-up of the peripheral part of the cell. The nuclei are spherical and have one large conspicuous nucleolus and usually one or more smaller nucleoli.

A mucous gland on keeping, changes in its behaviour to acetic acid, in so far that a piece of it teased-out in the dilute acid swells up more. Thus it may swell up into a jelly-like mass in 5 p.c. or even in stronger, acetic acid, although the fresh gland teased out in 1 p.c. acetic acid would have shown to the eye no swollen jelly-like mucin.

Mucous cells of the alimentary tract and of skin. The mucous cells which occur in the mucous membranes and skin of various animals have of late years received a good deal of attention.

I propose to state as briefly as possible the results of my own observations; at some later time I may discuss how far these agree or disagree with the observations of others, especially with those of Biedermann, Schiefferdecker, List, Merk and Paneth.

The cells of this kind on which I have made most observations are the cells of the mucous membrane of the œsophagus of the frog and salamander, and the epidermal cells of the earth-worm.

The superficial mucous cells of the œsophagus of the frog do not

usually show in the fresh state distinct granules, nor are they brought out by irrigation with salt solutions.

On the other hand the mucous cells of the œsophagus of the Salamander maculata do usually show distinct granules—15 to 20 in the length of the cell—and they are rendered more distinct by treatment with salt solutions. The salt solution causes the nuclei to swell up enormously. Reagents which bring out a network in the mucous cells of the salivary glands, do this also in the mucous cells of the salamander, but it is much less obvious and its amount is less. The granules may with care be preserved in osmic acid vapour[1], and be visible in sections of the œsophagus.

In the epidermis of the worm, both transparent goblet cells[2] and cells containing discrete granules of varying but generally large size, are present, in addition to the ordinary columnar cells with finely, and indistinctly granular cell-substance; the larger granular cells are in shape like the goblet cells (Claparède, Vejdowsky and others). In hardened specimens, cells are here and there seen like goblet cells except that they contain a few spheres like those of the granular cells. A small canal in the cuticle leads from both goblet and granular cells to the surface of the skin. Apparently granular cells give rise to goblet cells. When a worm is stimulated with interrupted shocks, it rapidly secretes a mucous substance[3], which by the movements of the worm becomes soon twisted into a white stringy mass around it; a portion of this when examined under the microscope does not show granules corresponding with the granules of the epidermic cells, spoken of above. The white stringy mucus does not swell up in cold water, although if the worm be placed in water and then stimulated, the mucous substance secreted forms a ropy mass; where this is exposed to the air a film of calcium carbonate is deposited owing to escape of carbonic acid. If the substance secreted on stimulating the worm be removed, and the worm placed in a little water, subsequent stimulation will give rise to a fresh secretion, although very much less than that first obtained. If a worm —or portion of a worm—be thus stimulated at intervals during an hour or two, until little or no secretion is obtained, and then be hardened, it

[1] For method cf. *Proc. Physiol. Soc.*, this *Journal*, Vol. x. No. 3, 1889.

[2] The goblet cells do not stain with reagents which stain the mucous cells of mammalian salivary glands, they show only a small amount of cell-substance, which in the outer part of the cell forms an irregular network.

[3] On stimulating a worm, a certain amount of a slightly milky fluid is extruded from the body cavity to the surface of the skin, this is crowded with leucocytes, contains a certain number of granules and cells from the layer around the intestine.

will be seen that both goblet cells and granular cells are much fewer in number. From the above mentioned facts it seems to me probable that the granules are turned out to form the mucous substance and that the goblet cells are the more or less emptied granular cells, but I have not been able to obtain completely satisfactory proof of this; the chief difficulty being the behaviour of the granules to reagents. They are readily preserved by osmic acid—and as Miss Greenwood has pointed out to me—by nearly all hardening agents. In the preserved specimens they all do not behave with reagents as do the mucous granules of the mammalian salivary glands, they are far more stable; for example after treatment for an hour or two with 1 p.c. osmic acid they do not disappear on irrigation with 1 p.c. potash.

I have made a few observations upon mucous cells in many other positions, and it appears to me that no common account is possible for them all. I am inclined to think that the most general description applicable to them is, that originally protoplasmic cells containing small proteid granules form spheres of mucous substance, these may occupy nearly the whole of the cell or leave free a basal portion. The spheres differ in their chemical characters, so that they may be more refractive than the cell-substance, or become so on treatment with salt solutions, or they may be of the same or nearly the same refractive index as the cell-substance and remain so on treatment with salt solutions. In some cases the mucous spheres increase at the expense of the finely granular protoplasm and run together, so that the protoplasm is then present as a network running through the mass of mucin. The amount of protoplasm left as a network varies in different cases, and it may be nearly, and possibly entirely, absent from the luminar portion of the cells. In other cases the spheres increase at the expense of the cell-substance, without running together, so that they are separated from one another for the most part by fluid and not by cell-substance.

Previous observations on Mucous Granules.

Heidenhain[1] in 1868 described the appearance of the fresh dog's sub-maxillary gland when teased-out in indifferent fluids. The cells were not distinctly separable from one another, they contained strongly refractive granules and vacuoles, and often large clear drops. In a

[1] *Studien des physiol. Institute zu Breslau*, p. 11.

more recent general account of mucous glands (1880), Heidenhain[1] speaks of the cells as being crowded with sharply contoured vacuole-like bodies. Heidenhain does not however discuss the nature of these bodies.

Lavdowsky[2] describes the mucous cells of the fresh gland as being coarsely granular and the demilune cells as being finely granular, but it is doubtful how much weight is to be attached to this description, since Lavdowsky speaks (*op. cit.* p. 297) of the granules seen in mucous cells after treatment with neutral ammonium chromate 5 p.c. as being the same as those seen in the fresh gland.

In 1879 I[3] described the granules of the fresh sub-maxillary gland of the cat and dog, and their disappearance from the outer region of the cells during secretion, and pointed out that they were probably homologous with the granules of the albuminous salivary glands; and in 1886 I gave a brief account[4] of some of the chief points of this paper.

There have been also a few observations on mucous cell granules in glands other than salivary glands.

In the mucous cells of the œsophageal glands of the frog distinct granules, forming an inner zone during secretion, were observed by Sewall and myself[5] in 1879. The granules were not distinguishable from granules of the pepsin-producing cells, until after the addition of reagents.

In 1882 Biedermann[6] gave an account of the mucous glands of the tongue of the frog. The cells during rest were crowded with conspicuous granules, and these disappeared from the basal part of the cell during secretion. Later (1886) Biedermann[7] found that in the mucous glands both of the tongue and of the nictitating membrane of the frog, the granules during secretion become changed into a much less refracting substance, thus making the cell appear as if vacuolated. According to Drasch[8], however, granular cells are not a constant feature of the mucous glands of the nictitating membrane of the frog, nor, when present, do the cells alter in granularity according to their state of secretion.

[1] *Handbuch d. Physiologie* (Herausg. v. Hermann) Bd. v. p. 93.
[2] *Archiv. f. mic. Anat.* Bd. 13, 1876.
[3] This *Journal*, Vol. II. pp. 276, 279.
[4] *Proc. Roy. Soc.* Vol. XL. No. 244, p. 362.
[5] This *Journal*, vol. II. p. 284.
[6] *Sitzungsb. d. kais. Akad. zu Wien*, Abt. III. Bd. 86.
[7] *Sitzungsb. d. kais. Akad. zu Wien.* Abt. III. Bd. 94.
[8] *Arch. f. Anat. u. Physiol.* Abt. 1889, p. 96.

Whilst granules have been so seldom described in mucous glands, they have not infrequently been described in some of the mucous cells of mucous membranes. A full account of the descriptions which have been given of these cells will be found in a recent paper by Paneth[1].

FIGURES. PLATE XXX.

Fig. 1. Alveoli of fresh sub-maxillary gland of dog, irrigated with 5 p.c. NaCl., showing mucous granules streaming out of an alveolus (*a*).

(*b*) Bleb of swollen, transparent mucin with granules in it.

(*c*) Other alveoli with mucous granules.

(*d*) Demilune cells with smaller, fainter proteid granules.

Magnified about 480 times.

Fig. 2. Alveolus of sub-maxillary gland of dog, showing the pseudo-network which is sometimes seen a short time after a piece of gland has been mounted.

Fig. 3. Alveolus of orbital gland of cat, in 5 p.c. NaCl, slightly pressed, showing the limits of the cells as clear lines.

Fig. 4. Isolated mucous granules of the orbital gland of the dog.

(*a*) Swollen in 1 p.c. NaCl.

(*b*) In 5 p.c. NaCl, showing faint filament sometimes seen to join and connect the granules, probably in consequence of their having stuck together.

(*c*) Shrunken in 20 p.c. NaCl.

Fig. 5. Isolated cells from sub-maxillary gland of dog, a day after death.

(*a*) Cells in 5 p.c. NaCl; two of the cells are focussed to show the nuclei, the nucleus is often completely hidden by the granules.

(*b*) Cells isolated in 5 p.c. NaCl, and then irrigated with 1 p.c. HCl.

(*c*) Demilunes in 5 p.c. NaCl.

Fig. 6 (*a*) Mucous cells sub-maxillary gland of dog irrigated with water, showing different sized network in the cells according to the amount they swell up; the network is here represented as more distinct than it usually is on addition of water. Nuclei rather refractive and homogeneous in appearance.

(*b*) Mucous cell of sub-maxillary gland of dog, isolated in 5 p.c. NaCl, and then irrigated with 1 p.c. HCl, showing fat globules in the meshes of the network.

[1] *Archiv für mik. Anatomie*, Bd. 31, p. 146, 1888.

(*c*) Mucous cell of sub-maxillary gland of cat, irrigated first with dilute ammonia, then with 1 p.c. HCl, showing bleb of transparent mucin issuing from the cell.

(*d*) Mucous cell of orbital gland of dog, two days after death. Isolated in 5 p.c. NaCl. and irrigated with 1 p.c. HCl, showing bulging of mucous substance between the bars of the network.

(*e*) Mucous cells from orbital gland of cat, one day after death. Isolated in 2 p.c. NaCl, a few granules only are left in the cells.

(*f*) Isolated nuclei of mucous cells of sub-maxillary gland of dog.

Fig. 7. Cells from orbital gland of dog left for three days in 2 p.c. chloral hydrate.

(*a*) Cell irrigated HNO_3 5 p.c., showing wide-meshed inner network.

(*b*) Cell irrigated with water, showing fine-meshed network on the surface of the cell.

Fig. 8. Ducts of sub-maxillary gland of the dog.

(*a*) Without addition of fluid the granules are not always so distinct as they are represented here, but may become more distinct on irrigation with 5 p.c. NaCl.

(*b*) Showing alteration which may take place a short time after the piece of gland has been mounted. Early stage of striation.

(*c*) Irrigation with water, granules swell up and give a vacuolated appearance to the duct cells.

June 28.

ON THE STIMULATION EFFECTS IN A POLARISED NERVE DURING AND AFTER THE FLOW OF THE POLARISING CURRENT.

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I PROPOSE here to consider somewhat more in detail the work of which a preliminary sketch was given in this Journal[1] some months ago. The object of the enquiry was to investigate the electrical changes which take place in a polarised nerve when it is stimulated, both during the flow and after the opening of the polarising current, so far as these changes have not hitherto been studied, or where the study of them has been incomplete. It was thought that if the gaps in our knowledge of this subject could be filled, the search for a principle by which to explain the phenomena, whose fundamental unity it is difficult to doubt, would be made at once more easy and more safe. I have tried to obtain not only qualitative but also quantitative results, relying in the latter case rather on comparison of different experiments on the same nerve, than on observations made on different nerves at different times. I shall endeavour here, as briefly as possible, to chronicle the results and to discuss them; to connect them with each other if I can; to connect them with already established facts if that be possible; and finally to suggest an explanation of them.

As far as possible, repetition of anything in the preliminary paper has been avoided. Accordingly, unless it has been first read, the present paper will in parts be scarcely intelligible.

I. GALVANOMETRIC OBSERVATIONS DURING THE FLOW OF THE POLARISING CURRENT, WHERE THE WHOLE INTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes in extrapolar region.*

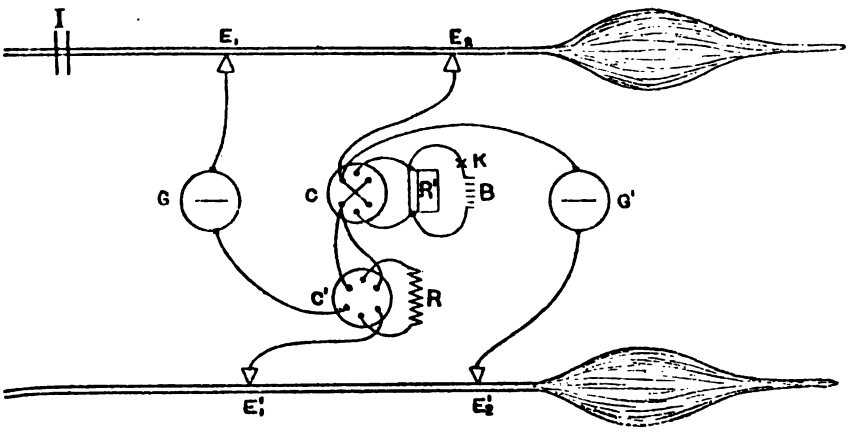
What I wished particularly to study here was the case where stimulation is made on the anodic side. Hermann found long ago

[1] Vol. IX. pp. 199—212.

that, when the excitation has to pass the cathode, the intrapolar effect falls out altogether, as soon as a certain limiting intensity of current is reached. He was unable to say whether, with stimulation on the side of the anode, the effect continually increases, or whether it reaches a maximum. For the deflection became so irregular, when the current was strengthened, that it was impossible to continue the observations. It was just, however, the strong currents which I was desirous of studying. In this difficulty a device occurred to me which has proved to be of very great service. I thought that, if the two nerves of the same frog were put one into each coil of a differential galvanometer, the current being divided between them, any irregularities in battery or nerves would be eliminated. The stimulation effect could then be got by exciting one of the nerves.

Fig. 1 shews the arrangement adopted.

FIG. 1.



I shews the position of the stimulating electrodes, which were 2 mm. apart; E_1 E_2, E_1' E_2' are the polarising electrodes; *G*, *G'* are the two galvanometer coils; *C* is a Pohl's commutator for changing direction of current; *C'* is a Pohl without cross wires by means of which a plug rheostat *R* could be introduced into either circuit. The circuit which did not contain *R* was completed at *C'* by a movable copper bridge.
R' is a rheochord, *B* is battery, *K* a key.

As it was not possible to adjust the nerves so that the resistance should be the same in both circuits, a rheostat was connected up in such a way that it could be placed in series with either of them. Then, with a very weak polarising current, the resistance was arranged so as to bring the image to zero of the scale. The intensity of the current would then be the same in both nerve circuits, if the galvanometer

constant of the two coils was the same and also the moment of the two magnets. At corresponding cross sections the current density would be approximately the same, and therefore whatever galvanometric change was caused in one nerve by the continued flow of the current would be balanced by an equal change in the other, except so far as accidental differences in the properties liable to be affected by the current are concerned. When the stronger current was now closed, the adjustment was still nearly right. The rheostat was again arranged so as to bring the image to zero, if the deflection was large. Then the stimulation effect was observed. In this way it was found possible to work with currents up to that of 7 or 8 Daniells with as much comfort, and nearly as great accuracy, as with 1 Daniell when a "Reductionsrolle" was used.

Experiments 1 and 2 are examples of the method.

EXPERIMENT 1.

Polarising current.	Stimulation effect.	Distance of coils of Induction machine.
1 D whole Rh ↑	+ 60	85
5 D whole Rh ↑	0	0
5 D whole Rh ↓	+ 55	0
1 D whole Rh ↓	+ 87	0
1 D whole Rh ↓	+ 55 ?	85
5 D ↓	+ 30	"
7 D ↓	+ 14	"
1 D whole Rh ↓	+ 85 / + 90	"

Length of nerve between electrodes 12·5 mm.

EXPERIMENT 2.

Polarising current.	Stimulation effect.	Stimulus.
1 D Rh 50 ↓	+ 25	85
1 D whole Rh ↓	+ 41	"
2 D ↓	+ 28	"
3 D ↓	+ 17	"
4 D ↓	+ 24	"
5 D ↓	0	"
1 D whole Rh ↓	+ 36	"

Distance I E_1 20 mm.
E_1 E_2 9 mm.

The whole rheochord contained 2000 rheochord units.

Before adopting this arrangement I had made a series of experiments in which the irregularity was diminished by shunting the galvanometer, the deflection being balanced by a "Reductionsrolle." The results here were not at all doubtful, although the method was inconvenient and not well suited for quantitative work. Experiments 3 and 4 may serve as examples. I do not lay much stress upon the actual numbers here, except with the unshunted galvanometer; and my conclusions are based mainly upon the results of the first method.

Experiment 3.

Polarising current.	Stimulation effect.	Stimulus.	
1 D ↑	+ 15	200	Shunt 10
1 D ↓	+ 65	"	"
3·2 D ↓	+ 50	"	"
1 D ↓	+ 70	"	"
1 D ↑	+ 12	"	"
4 D ↓	+ 15	"	"
1 D ↓	+ 75	"	"
4 D ↓	+ 12	"	"
9 D ↓	+ 2	"	"
9 D ↓	+ 5	120	"
1 D ↓	+ 70	200	"

Distance between polarising electrodes 11 mm.

Experiment 4.

Polarising current.	Stimulation effect.	Stimulus.	
1 D ↓	+ 160	200	No shunt
1 D ↑	+ 50	"	"
1·7 D ↑	0	"	Shunt 10
1·7 D ↓	+ 50	"	"
1·7 D ↑	0	"	"
2·6 D ↓	+ 12	"	"
2·6 D ↑	0	50	"
4 D ↓	0	200	"
4 D ↓	+ 50	70	"
3·2 D ↓	+ 5	200	"
	+ 25	120	"
2·6 D ↓	+ 4	200	"
	+ 25	120	"
1 D ↓	+ 5	200	"
	+ 55	120	"

Except when it is otherwise stated, the stimulating electrodes were at the central end of the nerve.

In all the experiments the numbers under the heading "Stimulus" refer to the distance between the coils of the induction machine expressed in millimetres. 1 D under "polarising current" means one Daniell. The sign prefixed to the numbers in the column headed "stimulation effect" gives the direction of the effect referred to that of the polarising stream, a + effect being one in the same direction, a − effect one in the opposite direction. In Experiments 3 and 4 the numbers representing the stimulation effect must be multiplied by the shunt number, in order to get the absolute deflection. In Experiments 1 and 2 the numbers must be doubled in order to compare with observations where the whole galvanometer was in circuit with one nerve, because here the stimulated nerve was connected up with only one coil. Again in 1 and 2 Helmholtz's wire was used, and there were two Daniells in the primary. In 3 and 4 there was only one Daniell in the primary, but the ordinary tetanising arrangement was employed. Even if the external conditions were made the same, it would not be so safe to compare the results from different nerves as to compare those from the same nerve. What is very evident from all the experiments, (of course those quoted are only a sample) is that with

stimulation on the anodic side a limiting intensity of current can be reached for which the stimulation effect disappears altogether. This lies much higher than the limiting intensity for stimulation on the cathodic side. It is raised by strengthening the stimulus in both cases, but this is much more marked for anodic than for cathodic stimulation. The longer the piece of nerve between the polarising electrodes the greater, in general, must the E. M. F. be with which the limit is reached; in other words the current density is the function of the current which is chiefly concerned. Experiment 3 shews very clearly that the decline of the effect as the current is increased is not due to permanent injury to the nerve, for with 1 D ↓ the effect is rather greater at the end than at the beginning. That the decline is not due to the deepening of anelectrotonus at the stimulated point, we shall be able to shew later on, when we come to speak of the comparison of the extra- and intrapolar effects. To eliminate this possibility, as far as might be, the stimulating electrodes were always placed at as great a distance from the polarising circuit as the conditions of the experiment allowed.

The curve of the stimulation effect, then, rises from a zero value and sinks to a zero value again. Between the two points where it cuts the abscissa axis it must have at least one maximum. I tried to determine the current density corresponding to which the anodic curve reaches its maximum; and it came out that this current density cannot lie very far from that for which the cathodic curve cuts the abscissa. It will be seen, that in the experiments already cited the highest values of the stimulation effect for the descending current always correspond to zero, or something very like zero, for the ascending current. Thus in Experiment 3, 1 D ↓ gives the largest stimulation effect; 1 D ↑ gives one which is so much smaller that a slight increase in the current would have brought us to the zero. In Experiment 4, 1·7 D ↓ corresponds to the maximum and 1·7 D ↑ to the minimum. Experiment 1 was not directed to this point, and there is too great a gap between 1 D and 5 D. What we see is that with 1 D whole Rh ↑ the cathodic curve is already lower than the anodic, (leaving out of account the doubtful observation) which is higher than with any of the stronger currents.

Experiment 5 is an example of observations specially devoted to this point. Experiment 6 compares the effect with the two directions for currents which were too weak to produce a complete block at the cathode.

Experiment 5.		Experiment 6.		Experiment 7.	
Polarising current.	Stimulation effect.	Polarising current.	Stimulation effect.	Polarising current.	Stimulation effect.
1/9 D ↓	+ 20	·075 D ↓	+ 15	1/25 D ↓	+ 50
↑	+ 18	↑	+ 16	↑	+ 20
1 D ↓	+ 130	·25 D ↓	+ 35	8 D ↓	0 } even with strongest stimulation.
↑	+ 46	↑	+ 36		
2 D ↓	+ 130	·575 D ↓	+ 83	8 D ↑	0
↑	0	↑	+ 52		
2 D ↓	117	·75 D ↓	+ 99		
↑	0	↑	+ 57		
		1 D ↓	+ 97		
		↑	+ 64		

Experiment 7 shews that in a perfectly fresh nerve the limit was certainly not higher than 8 Daniells for the descending current.

2. *Stimulating electrodes in intrapolar region.*

We have seen that there is a limit beyond which extrapolar stimulation on the side of the anode evokes no response in the intrapolar area, any more than stimulation on the side of the cathode. The block at the cathode we know to be a polar effect, because stimulation on the side of the anode is as effective as before. Besides, there is plenty of direct proof that the intrapolar area as a whole has not lost its excitability and conductivity, when the cathode has become impassable. The question, therefore, arises, Is the final disappearance of the effect due to a polar anodic block, or is it due to a depression of conductivity in the whole intrapolar region, extending also into the nearest extrapolar parts? If the depression is limited to the region immediately around the anode, there is no reason why the intrapolar effect should not persist with intrapolar stimulation long after it has ceased with extrapolar. This assumes that excitation of the intrapolar area generally is the essential condition of the stimulation effect. If the essential condition be a difference in the intensity of excitation at the two poles, in other words if polarisation increment be the main factor, then indeed intrapolar stimulation might be effective after the block was established at both poles, and yet there might be no change indicated by a

galvanometer connected up with the whole intrapolar area in the usual way. We shall see later on that there is evidence to shew that the anodic block is not essentially a polar effect, as the cathodic block is, but that it is really a part of the general intrapolar depression. The very gradual decline of the effect is already a hint of this.

Arrangements. It was necessary to introduce a large resistance into the secondary coil, so as to prevent the polarising current from being short-circuited through the stimulating electrodes. A plumbago resistance of 578,000 ohms (resistance B) served this purpose effectually. Stimulation was made by closing the primary circuit, the du Bois Key in the secondary remaining open. This was to avoid any error due to the slight alteration in the total resistance of the nerve circuit which would have been caused by throwing in the secondary coil. As a rule it was not found necessary to introduce extra resistance into the nerve circuit. When it was thought necessary, a plumbago resistance of about 68,000 ohms, which will be referred to as A, was introduced. Even when there was no extra resistance in the nerve circuit, fairly strong shocks could be employed without affecting the galvanometer directly. Helmholtz's modification was used as before. The differential galvanometer arrangement was that adopted. It is unnecessary to give a figure, as everything was precisely the same as in Fig. 1, except that the stimulating electrodes were intrapolar. Except where it is otherwise stated, they were as nearly as possible in the middle of the intrapolar region.

Experiment 8.		Experiment 9.		
Polarising current.	Stimulation effect.	Polarising current.	Stimulation effect.	
1 D Rh 100 ↑	+ 26	1 D Rh 15 ↓	+ 23	
1 D whole Rh ↑	+ 48	↑	+ 30	Distance between polarising electrodes 14 mm.
1 D ↑	+ 60	1 D Rh 100 ↓	+ 42	
2 D ↑	+ 23	↑	+ 44	Cross section of nerve between electrodes nearly uniform.
3 D ↑	+ 21	1 D ↓	+ 71	
4 D ↑	+ 13	1 D ↑	+ 69	
5 D ↑	+ 5	1 D Rh 15 ↓	+ 20	

EXPERIMENT 10. Here two pairs of nerves were used. The central end of one nerve was put in contact with the peripheral end of the other so as to equalize the cross section. The direction of the current is reckoned with reference to the nerve whose central end is next electrode E_1.

Polarising current.	Stimulation effect.	
1 D whole Rh ↑	+ 78	
↓	+ 71	
↑	+ 86	
↓	+ 78	Stimulus 25; 2 D in primary; distance between polarising electrodes, 17 mm.
2 D ↓	+ 50	
↑	+ 55	
3 D ↑	+ 14	
↓	+ 15	
4 D ↓	+ 16	

These experiments shew very much the same state of things, as when stimulation is extrapolar and above the anode. The effect rises at first to a maximum, and then gradually declines to zero. In Experiment 8 5 D ↑ gives very nearly the limiting current density. The maximum lies about 1 D, certainly much below 2 D. There is no hint of an indefinite persistence of the effect beyond the current density which limits it for extrapolar stimulation.

When we come to the comparison of the extra- and intrapolar effect with intrapolar stimulation, we shall find additional evidence to shew that the anode becomes impassable about the time when the intrapolar area in general has lost its conductivity and excitability. On the other hand it is seen that, long after the cathodic block has developed itself, intrapolar stimulation is effective, producing indeed something like its maximum effect with the current density which corresponds to the beginning of the block.

II. GALVANOMETRIC OBSERVATIONS DURING THE FLOW OF THE POLARISING CURRENT WHEN AN EXTRAPOLAR AREA IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes extrapolar.*

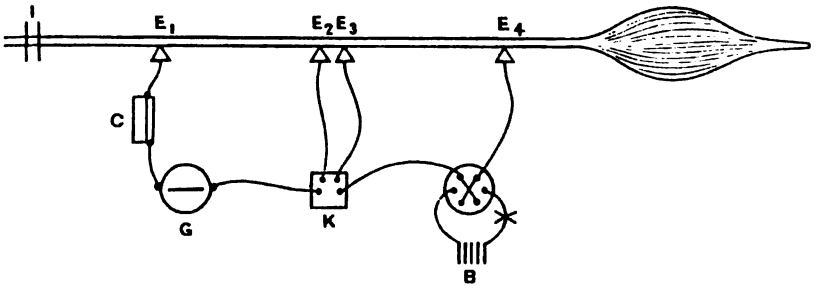
Since Bernstein first discovered the electrotonic variation, the subject has been more than once investigated, by Hermann among others. Hermann explained the diminution which takes place alike in the anodic and cathodic currents when the nerve is stimulated, by an

application of his law of polarisation increment[1]. Later on, however, he was convinced by rheotome experiments that, although this might be part of the explanation, it was not the whole, and he supplemented it by assuming that excitation diminishes the capacity for polarisation between core and sheath[2].

The results of my experiments on the intrapolar effect made me anxious to use stronger currents, and to investigate the direction of the extrapolar effect especially on the side of the anode. For it was to be expected that, as soon as the anodic block was established, a resultant action current[3] must be caused between any extrapolar point and the anode itself, or a point near the anode. This current must have the opposite direction to the negative electrotonic variation, and, if it were large enough to overcome the latter, it ought to appear as a positive variation. On the side of the cathode, however, it would have the same direction as the electrotonic variation, and would only tend to strengthen this. I kept also the possibility in view that the true electrotonic variation itself, whatever its cause, might not necessarily have the same direction with strong currents which rendered the intrapolar region non-conducting, as with weak.

Since it was to be expected that the extrapolar parts of the nerve in the immediate neighbourhood of the anode would have their conductivity most reduced, the indication was to approach the lower

FIG. 2.



E_1 E_2 are the galvanometer electrodes; E_3 E_4 polarising electrodes.
G is the galvanometer. C the compensator. B the battery.
K a double key by which battery and galvanometer circuits were closed simultaneously.

leading-off electrode as near as possible to the upper of the polarising pair. The electrotonic currents, which were very large, were carefully

[1] Pflüger's *Archiv*, Bd. VI., and X. p. 215.

[2] Pflüger's *Archiv*, Bd. XLII. p. 246, &c.

[3] By resultant action current is meant the algebraic sum of all the action currents set up in a given interval. The galvanometer would practically carry out the integration.

compensated in order to eliminate the ordinary positive variation, or "change of resistance" effect, which could not otherwise be taken account of. To make sure that this compensation did not essentially alter the conditions determining the spread of the electrotonic currents, a separate set of observations was taken where there was no compensation. Fig. 2 shews the arrangement at first used, details being omitted.

EXPERIMENT 11.

Polarising current.	Stimulation effect.	
1 D ↓	− 60	distance of E_2 from E_3 1½ mm.
5 D ↓	+ 125	"
5 D ↑	− 58	"
5 D ↓	− 12	distance of E_2 from E_3 8 mm.
5 D ↑	− 19	

Stimulus, Helmholtz wire, distance of coils 85.

Distance between I and E_1 8 mm.
" " E_1 and E_2 9 mm.
" " E_3 and E_4 9 mm.

EXPERIMENT 12.

Polarising current.	Stimulation effect.	
1 D ↓	− 26	
2 D ↓	+ 25	current had been 4′ closed.
3 D ↓	+ 62	
4 D ↓	+ 135	
5 D ↓	+ 138	
7 D ↓	+ 126	
10 D ↓	+ 106	
4 D ↓	+ 115	

Stimulus 85; I E_1 9 mm.
E_1 E_2 9 "
E_2 E_3 1½ "
E_3 E_4 9 "

EXPERIMENT 13.

Polarising current.	Stimulation effect.
1 D ↑	− 19
2 D ↑	− 33
3 D ↑	− 37
5 D ↑	− 45 ?
10 D ↑	− 44

The nerve was the companion nerve of that used in Experiment 12. Distances the same as in Experiment 12.

EXPERIMENT 14.

Polarising current.	Stimulation effect.	
1 D ↓	− 45	distance I E_1 8 mm.
2 D ↓	− 73	E_1 E_2 9 "
3 D ↓	− 68	E_2 E_3 7 "
1 D ↓	− 30	E_3 E_4 9 "
5 D ↓	− 47	
7 D ↓	− 27	
7 D ↓	− 28	without compensation.
7 D ↓	− 37	distance E_2 E_3 3 mm.
1 D ↓	− 5	
1 D ↓	− 26	distance E_2 E_3 1 mm.
3 D ↓	+ 34	
5 D ↓	+ 26	

Only one coil of galvanometer used in this experiment.

EXPERIMENT 14 a.

Polarising current.	Stimulation effect.	
1 D Rh 1000 ↑	− 85	
	− 55	45″ after opening.
	− 36	2′ „
	− 17	4′ „
	− 3	6′ „
1 D Rh 100 ↑	+ 6	30″ after closure.
	− 18	in 1′ more } Here the current was allowed to flow, the stimulation effect being read at intervals.
	− 22	1′ „
	− 28	1′ „
	− 35	1′ „
	− 38	1′ „
	− 43	15″ after opening.
2 D ↑	− 106	
	− 129	30″ after opening.
	− 114	1′ more.
	− 91	1′ „
	− 68	2′ „
	− 57	2′ „
	− 53	2′ „
	− 51	2′ „
3 D ↑	− 95?	
	− 117	20″ after opening.
	− 86	1′ more.
	− 68	2′ „
	− 62	1′ „
3 D ↓	+ 235	
closed for 5′	+ 48	30″ after opening.
	+ 2	1′ more.
	− 17	1′ „
	− 42	2′ „
	− 46	6′ „

Stimulus 14. Distance $I\,E_1$ 11 mm.
$E_1\,E_2$ 9 „
$E_2\,E_3$ 0 „ i.e. E_2 and E_3 united.
$E_3\,E_4$ 9

"After opening" means after opening the polarising current.

EXPERIMENT 14 b.

Polarising current.	Stimulation effect.	
1 D ↑	– 98	
	– 47	30″ after opening.
	– 42	1′ ,,
	– 31	2′ ,,
	– 21	4′ ,,
	– 17	6′ ,,
1 D ↑	– 112	
	– 118	15″ after opening.
1 D ↑	– 127	
	– 97	20″ after opening.
	– 53	5′ ,,
	– 51	6′ ,,
2 D ↑	– 67	
	– 74	30″ after opening.
2 D ↑	– 62	
	– 65	25″ after opening.
	– 55	7′ ,,

Stimulus 14. Distance $I\ E_1$ 12 mm.
$E_1\ E_2$ 11 ,,
$E_2\ E_3$ 0 ,,
$E_3\ E_4$ 9 ,,

EXPERIMENT 14 c.

Polarising current.	Stimulation effect.	
1 D 200 Rh ↑	– 24	
	– 39	10″ after opening.
	– 28	1′ ,,
	– 20	2′ ,,
	– 10	7′ ,,
1 D Rh 100 ↑	– 18	
	– 13	20″ after opening.
	0	2′ ,,
1 D Rh 50 ↑	– 10	
	– 4	15″ after opening.
	0	1′ ,,
1 D ↑	– 58	
	– 65	35″ after opening.
	– 34	in 2′ more.

Stimulus 14. Distance $I\ E_1$ 11 mm.
$E_1\ E_2$ 9 ,,
$E_2\ E_3$ 0 ,,
$E_3\ E_4$ 9 ,,

Experiments 15 and 16 are examples of the experiments where the electrotonic currents were not compensated.

EXPERIMENT 15.

Polarising current.	Stimulation effect.
1 D Rh 100 ↓	− 3
↑	− 3
1 D whole Rh ↓	− 25
↑	− 20
1 D ↓	− 20
↑	− 12
1 D ↓	− 15
3 D ↑	− 55
3 D ↓	+ 50
5 D ↑	− 45
5 D ↓	+ 53

Distance $I\,E_1$ 9 mm.
$E_1\,E_2$ 11 „
$E_2\,E_3$ 2 „
$E_3\,E_4$ 9 „
Galvanometer Shunt 10.
Helmholtz wire not used.
Stimulus much stronger than in Experiments 11—14.

EXPERIMENT 16.

Polarising current.	Stimulation effect.	
1 D Rh 10 ↑	− 28	
↓	− 55	
1 D Rh 100 ↓	− 17	Shunt 10
↑	− 3	„
1 D ↓	− 15	„
3 D ↓	+ 55	„
3 D ↑	− 30	„
2 D ↓	− 14	„
3 D ↓	+ 65	„
3 D ↑	− 16	„

Distance $I\,E_1$ 15 mm.
$E_1\,E_2$ 14 „
$E_2\,E_3$ 1½ „
$E_3\,E_4$ 9 „

To get rid of the unsteadiness of the deflection, which is especially troublesome in the case of the anodic current, I had recourse again to a method of what might be called automatic compensation, where the electrotonic currents of two nerves were made to oppose each other, the balance being completed by means of a compensator. Figs. 3 and 4 are simple schemes of two ways in which this was done. In the arrangement of Fig. 3 the nerves rested each on four electrodes. The distances $E_3\,E_4$ and $E_3'E_4'$ were made as nearly as possible equal, and the current from the same battery was divided between the two nerves. In the arrangement of Fig. 4 the nerves lay on the same pair of battery electrodes E_3 E_4. E_2 was a movable bridge of clay, which the nerves touched just where they began to diverge. In both cases the distances $E_1\,E_2$, $E_1'\,E_2'$ were made equal. It is clear, therefore, that in the galvanometer circuit the two electrotonic currents would nearly counterbalance each other. Any irregularities due to the battery would thus be eliminated. As it was not possible to hit the balance exactly, a compensator C was introduced to complete it. In one set of experiments two pairs of nerves in reverse

order were used, instead of two single nerves, in order to get a uniform cross section. When now one of the nerves or nerve pairs was stimulated, the stimulation effect would appear pure, but weakened by having to pass through the resistance of the included piece of unstimulated nerve. The deflection would have to be multiplied by 2 in order to make it comparable with the case where the galvanometer was connected only with the stimulated nerve itself. Here the resistance of the galvanometer is neglected.

I quote a few of the results got by this method, which has proved very satisfactory. Experiment 17 is an example with the arrangement

Fig. 3.

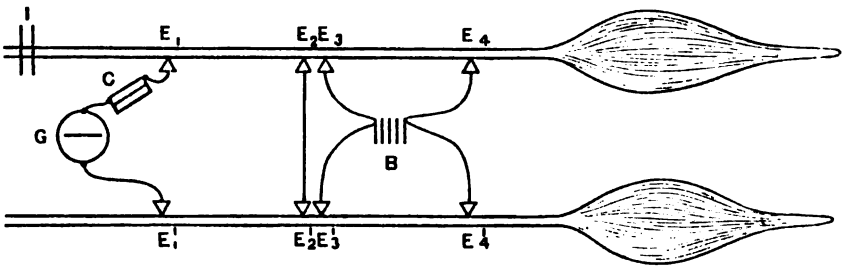
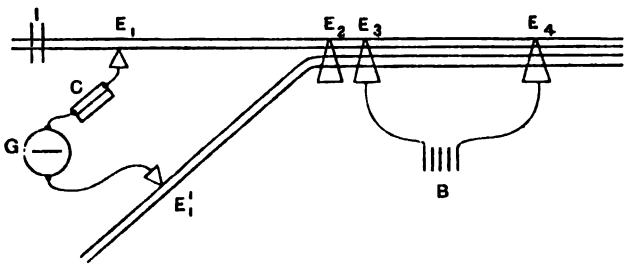


Fig. 4.



of Fig. 3. Experiments 18 to 22 were done with the arrangement of Fig. 4.

EXPERIMENT 17.

Polarising current.	Stimulation effect.	
1 D ↑	− 20	
3 D ↑	− 32	
4 D ↑	− 20	
	− 19	1′ after opening.
5 D ↑	− 24	
	− 38	30″ after opening.
	− 38	1′ „
	− 27	2′ „
5 D ↓	+ 34	
	+ 17	30″ after opening.
	+ 9	1½′ „

Distance $I E_1$ 9 mm.
$E_1 E_2$ 12 „
$E_2 E_3$ 0 „
$E_3 E_4$ 12 „

EXPERIMENT 18.

Polarising current.	Stimulation effect.	
1 D ↓	− 184	
	+ 16	20″ after opening.
3 D ↓	− 38	
	+ 46	20″ after opening.
5 D ↓	+ 58	
	+ 56	20″ after opening.
	+ 17	2′ „
8 D ↓	+ 68	
	+ 77	20″ after opening.
	+ 53	2′ „
	+ 26	6′ „
1 D ↓	− 138	
	+ 27	20″ after opening.
1 D Rh 100 ↓	− 53	
	+ 12	15″ after opening.
5 D ↓	+ 30	
	+ 35	20″ after opening.
8 D ↓	+ 63	
	+ 41	40″ after opening.
1 D ↓	− 79	
2 D ↓	− 76	

Distance $I E_1$ 10 mm.
$E_1 E_2$ 10 „
$E_2 E_3$ 2 „
$E_3 E_4$ 13 „
Stimulus 14.

EXPERIMENT 19.

Polarising current.	Stimulation effect.	
1 D Rh 50 ↑	− 9	
1 D Rh 200 ↑	− 5	
1 D ↑	− 14	
2 D ↑	− 27	
	− 5	20″ after opening.
3 D ↑	− 36	
	− 21	20″ after opening.
5 D ↑	− 21	
(closed 4′)	− 21	20″ after opening.
5 D ↓	+ 86	
(closed 4′)	+ 24	20″ after opening.
	+ 28	1′ ″
	+ 2	5′ ″
5 D ↓	+ 65	
(closed 3′)	+ 22	20″ after opening.
1 D ↓	− 42	
1 D Rh 200 ↓	− 39	

Distance $I\,E_1$ 8 mm.
$E_1\,E_2$ 9 ″
$E_2\,E_3$ 1 ″
$E_3\,E_4$ 13 ″
Stimulus 14.

EXPERIMENT 20.

Polarising current.	Stimulation effect.	
1 D ↓	− 33	E_2 and E_3 in contact.
3 D ↓	+ 33	
1 D ↓	− 53	distance $E_2\,E_3$ 3 mm.
3 D ↓	+ 5	
5 D ↓	+ 32	
1 D ↓	− 35	distance $E_2\,E_3$ $4\frac{1}{2}$ mm.
3 D ↓	− 24	
5 D ↓	− 19	
10 D ↓	− 1 (20″ after opening + 15)	

Distance $I\,E_1$ 8 mm.
$E_1\,E_2$ 9 ″
$E_3\,E_4$ 12 ″
Through whole experiment. Stimulus 14.

EXPERIMENT 21.

Polarising current.	Stimulation effect.	
1 D ↑	− 16	distance E_2 E_3 4½ mm.
2 D ↑	− 23	
1 D ↑	− 13	E_2 E_3 3 mm.
3 D ↑	− 18	
5 D ↑	− 23	
1 D Rh 100 ↑	− 7	E_2 and E_3 in contact.
2 D ↑	− 7	
5 D ↑	− 8	
	(20″ after opening − 7)	
8 D ↑	− 3	
1 D ↓	− 48	
3 D ↓	− 93	
8 D ↓	+ 58	
5 D ↓	+ 26	

Distances same as in Experiment 20.
Stimulus 14.

EXPERIMENT 22.

Polarising current.	Stimulation effect.	
2 D ↑	− 40	E_2 and E_3 very close but not touching.
1 D Rh 100 ↑	− 8	
3 D ↑	− 28	
5 D ↑	− 23	
	− 26	20″ after opening.
1 D ↑	− 9	E_2 and E_3 in contact.
2 D ↑	− 14	
5 D ↑	− 3	
	− 13 (20″ after opening)	
1 D ↓	− 120	
2 D ↓	+ 25	
5 D ↓	+ 122	
	+ 12 (20″ after opening)	
1 D ↓	− 47	

Distance I E_1 7 mm.
E_1 E_2 7 „
E_3 E_4 11 „ Stimulus 14.

It will be seen from these results, that with currents of only moderate strength the electrotonic variation is negative both on the side of the anode and on that of the cathode, however close E_2 and E_3 may be, and even when they are united. As the current is strengthened, the negative variation on the anodic side gives place to a positive variation, and this is reached with greater difficulty the greater the distance between E_2 and E_3. On the cathodic side, however, the variation remains negative with the strongest currents I have used. I have, indeed, in a few instances observed a small positive variation of the cathodic current, with perfectly fresh nerves and a weak polarising stream. In Experiment 14 a, e.g., with 1 D Rh 100 ↑, a deflection of + 6 was got at first. But, when the current was left closed for a longer time, this changed into a negative effect, which still increased with the time. In this connection one thinks of the fact that a weak current may establish the block at the cathode when it flows for some time, although it would not be able to do so with a shorter time of closure. The point will be referred to again.

Some observations were taken (Experiments 14, 14 a, 14 b, 14 c, 15, 17 and 18) to compare the amount of the stimulation effect during the flow of the current with the amount a very short time after opening it. The sign of the after-effect is seen to be positive on the side of the anode, negative on the side of the cathode. This is true whether the sign of the effect during the flow on the anodic side be positive or negative.

In general it will be seen that, even 20″ after opening the polarising current, the after-effect on the cathodic side is greater than the effect during the flow, while on the side of the anode this relation is reversed. Even where this does not hold for the cathodic side, and this is almost exclusively the case for the weaker currents, the after-effect in general bears a larger proportion to that during the flow than it does on the anodic side. The meaning of this we shall try to interpret later on.

2. *Stimulating electrodes in intrapolar region.*

Here it was necessary, as before, to have the large resistance B in the circuit of the secondary coil. The stimulating electrodes, which were, of course, non-polarisable, were generally placed in the middle of the intrapolar area. Otherwise the arrangements were precisely like those for extrapolar stimulation. Experiments 23 and 24 are examples of these done according to the scheme of Fig. 2, with the stimulating electrodes intrapolar.

EXPERIMENT 23.

Polarising current.	Stimulation effect.	
1 D ↓	− 53	Stimulus 25
1 D ↑	− 8	"
1 D Rh 16 ↓	− 4	"
1 D Rh 100 ↑	0	"
1 D Rh 400 ↑	0	"
↓	− 32	"
1 D ↑	0	"
2 D ↑	− 6	"
2 D ↓	− 15	"
4 D ↓	0	"
4 D ↓	− 14	Stimulus strongest
4 D ↑	− 11	"
6 D ↑	0	"
1 D ↓	− 63	Stimulus 25
1 D ↑	0	

Distance $E_1 E_2$ 12 mm.
$E_2 E_3$ 4 "
$E_3 E_4$ 25 "
Stimulating electrodes 2 mm. apart and in middle of intrapolar region.

EXPERIMENT 24.

Polarising current.	Stimulation effect.
1 D ↑	0
1 D Rh 100 ↑	− 14
1 D Rh 400 ↑	− 11
1 D whole Rh ↑	0
2 D ↑	0
3 D ↑	0
3 D ↓	− 22
5 D ↑	0
1 D ↓	− 89

Distance $E_1 E_2$ 9 mm.
$E_2 E_3$ 8 "
$E_3 E_4$ 20 "
Stimulating electrodes 2 mm. apart and in middle of intrapolar region.
Stimulus 25.

Experiments 25—27 were done by the method of Fig. 4, with stimulating electrodes intrapolar.

EXPERIMENT 25.

Polarising current.	Stimulation effect.	
1 D ↓	− 20	Stimulus 85
2 D ↓	− 83	Stimulus 10
3 D ↓	− 21	"
4 D ↓	− 18	"
5 D ↓	0	"
6 D ↓	0	"
3 D ↓	− 49	"
4 D ↓	− 24	"
5 D ↓	+ 4 (after 2′ flow; 0 at first)	"
8 D ↓	− 15	"
5 D ↓	+ 3	"

Distance $E_1 E_2$ 8 mm.
$E_2 E_3$ 3 "
$E_3 E_4$ 24 "
Stimulating electrodes in middle of intrapolar region.

EXPERIMENT 26.

Polarising current.	Stimulation effect.	
1 D ↓	− 37	
2 D ↓	− 46	
3 D ↓	− 69	
4 D ↓	− 12	
5 D ↓	+ 5	at first
	− 9	later
6 D ↓	− 7	
8 D ↓	0	
2 D ↓	− 13	
1 D ↓	− 72	
2 D ↓	− 24	
4 D ↓	+ 3	
1 D ↓	− 79	
6 D ↓	0	

Distance $E_1 E_2$ 12 mm.
$E_2 E_3$ 21/2 "
$E_3 E_4$ 22 "
Stimulating electrodes in middle of intrapolar region.
Stimulus 25.

EXPERIMENT 27.

Polarising current.	Stimulation effect.	
1 D Rh 100 ↑	− 5	Distance $E_1 E_2$ 10 mm.
1 D Rh 50 ↑	− 1	$E_2 E_3$ 0 ,,
1 D Rh 100 ↓	− 8	$E_3 E_4$ 23 ,,
1 D Rh 300 ↑	+ 3	Stimulating electrodes in middle of intrapolar region.
1 D Rh 300 ↓	− 34	Stimulus 25.
1 D ↑	0	
1 D ↓	− 75	
2 D ↑	0	
2 D ↓	− 55	
2 D ↑	0	
3 D ↓	− 16	
3 D ↑	0	
5 D ↓	0	
1 D ↓	− 39	
1 D ↑	0	
1 D Rh 50 ↑	− 5	
1 D Rh 16 ↓	− 14	
1 D Rh 16 ↑	− 2	

The chief points of difference between these results and those with extrapolar stimulation are (1) the almost complete absence of any stimulation effect on the side of the cathode except with the weakest currents, and (2) the absence of the positive effect on the anodic side. The first difference is without doubt due to the cathodic block. When the excitation is unable to pass the cathode the negative variation of the cathodic electrotonic current is absent, or present only in very small amount. Plainly, therefore, the important condition for this variation is stimulation of the extrapolar area itself. It is not enough that the intrapolar region, and even the extrapolar anodic region, should be excited. This is of considerable interest in connection with the explanation of the electrotonic variation put forward by Hermann, and already alluded to. It is not easy to see how a change in the polarisation constant in the intrapolar area should be unaccompanied by an effect on the cathodic electrotonic current, merely because of the block at the cathode. In the case of the intrapolar stimulation effect we know that nothing which happens in the extrapolar area has any influence, unless the excitation is able to pass the cathode. These things, on the face of them at least, seem to suggest that electrotonic variations, like other stimulation effects, only appear in parts of a nerve which are actually excited. There does not

seem any reason why this should be so, if the variations are due to alteration in the polarisation between sheath and core. But it is not so easy to apply the theory to this case as it is to another, which we shall come to and of which it seems to give a ready explanation. Besides it would be absurd to lay much stress on a few experiments which only incidentally raise the question. As to the second point of difference, the absence of the positive effect on the anodic side, we see that instead of changing sign with increasing current the effect declines from its maximum to zero. This is true even when one electrode is made common to the galvanometer and battery circuits, as in Experiment 27. The positive variation is got by exciting the anodic region when the anode is impassable. It appears, therefore, for extrapolar but not for intrapolar stimulation. This we shall see more clearly from the next section.

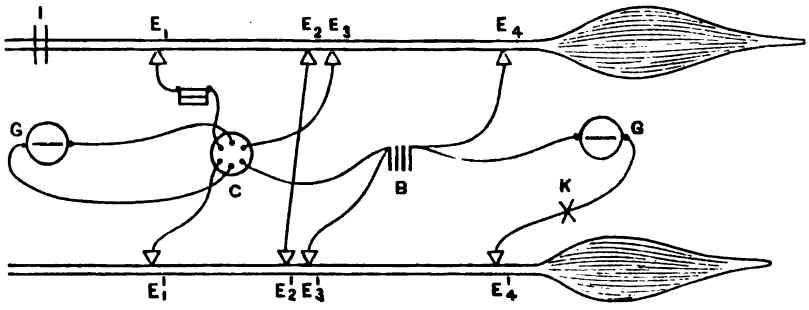
III. Observations during the flow of the polarising current when the whole intrapolar area is led off to the galvanometer alternately with an extrapolar area.

The object of this set of experiments was to compare directly, in the same nerve or nerve pair, the stimulation effect in the extra- and intrapolar regions.

1. *Stimulating electrodes extrapolar.*

Fig. 5 shews the arrangement generally used. By means of the commutator C, the second coil of the galvanometer G could be put into

Fig. 5.



C is a commutator by which one coil of the galvanometer G could be introduced into the extrapolar area E_1 E_2 or into the intrapolar area E_2 E_4. G' the other galvanometer coil is in circuit with the intrapolar region E_3' E_4' when the intrapolar effect is being measured, but the coil is short circuited by a du Bois key K when the extrapolar effect is taken.

circuit with the extrapolar or intrapolar area of the stimulated nerve at will. The other coil was only used for the intrapolar effect.

EXPERIMENT 28.

	Polarising current	Stimulation effect.
Extrapolar	5 D ↓	+ 180
Intrapolar	5 D ↓	0
Fresh nerve		
Extrapolar	4 D ↓	+ 214
Intrapolar	4 D ↓	+ 50
Extrapolar	4 D ↓	+ 81
Intrapolar	4 D ↓	+ 55
Extrapolar	4 D ↓	+ 170

Distances $I E_1$ 9 mm.
$E_1 E_2$ 8 „
$E_2 E_3$ 1 „
$E_3 E_4$ 9 „
Stimulus 85.

EXPERIMENT 29.

	Polarising current.	Stimulation effect.
Intrapolar	4 D ↓	0
Extrapolar	4 D ↓	+ 38
	4 D ↓	+ 57
Intrapolar	4 D ↓	+ 5
Extrapolar	4 D ↓	+ 39

Here only one coil of galvanometer in circuit of stimulated nerve. Distances and stimulus same as in Experiment 28.

In Experiment 29 the differential galvanometer method was used for the intrapolar observations, and one coil of the galvanometer for the extrapolar. The numbers would need to be multiplied by 2 in order to compare with Experiment 28 where the whole galvanometer was used. In Experiment 28 it will be seen that with 5 D ↓ the intrapolar effect is suppressed, while the extrapolar effect is not much less than with 4 D ↓. In Experiment 29 4 D ↓ is sufficient to give the limit. Put briefly, the maximum of the positive extrapolar effect, on the anodic side, with extrapolar stimulation is reached with a current which does not differ much from that which gives the minimum (zero) for the intrapolar effect.

On the side of the cathode the negative extrapolar effect may apparently increase somewhat after the intrapolar effect has disappeared; but the results were a little irregular.

2. *Stimulating electrodes intrapolar.*

Except in the position of the stimulating electrodes, the arrangement was the same as in Fig. 5.

Experiment 30.

Polarising current.	Intrapolar effect.	Extrapolar effect.
1 D whole Rh ↓	+ 137	− 37
2 D ↓	+ 55	− 21
3 D ↓	+ 12	− 5
1 D ↓		− 34
4 D ↓	+ 4	0
4 D ↓	0	
1 D whole Rh ↓	+ 131	− 44
1 D Rh 1000 ↓	+ 154	− 39
1 D Rh 500 ↓	+ 174	− 46
1 D Rh 200 ↓	+ 177	− 50
1 D Rh 100 ↓	+ 167	− 45
1 D Rh 16 ↓	+ 124	− 34

Experiment 31.

Polarising current.	Intrapolar effect.	Extrapolar effect.
unpolarised		+ 19
1 D Rh 100 ↑	+ 117	+ 5
1 D Rh 200 ↑	+ 152	+ 6
1 D 500 ↑	+ 215	+ 7
1 D Rh 1000 ↑	+ 213	+ 5
1 D whole Rh ↑	+ 291	+ 8
1 D ↑	+ 247	+ 7
2 D ↑	+ 281	+ 5
3 D ↑	+ 200	
4 D ↑	+ 44	

Experiment 32.

Polarising current.	Intrapolar effect.	Extrapolar effect.
Distance E_2 E_3 6 mm.		
1 D whole Rh ↓	+ 74	− 17
1 D ↓		− 21
2 D ↓		− 7
3 D ↓		− 5
1 D Rh 1000 ↓		− 23
1 D Rh 500 ↓		− 24
1 D Rh 100 ↓		− 16
Distance E_2 E_3 0 mm.		
1 D whole Rh ↓		− 31
1 D Rh 1000 ↓		− 27
1 D ↓		− 21
2 D ↓		− 10
4 D ↓		− 4

Distance E_1 E_2 17 mm.
E_3 E_4 25 „

Experiment 33.

Polarising current.	Intrapolar effect.	Extrapolar effect.
		unpolarised 2 r
1 D Rh 16 ↑	+ 7	
1 D Rh 100 ↑	+ 29 } + 37 }	4 r
1 D whole Rh	+ 84	8 r
1 D ↑	+ 103	
		unpolarised 3 r
2 D whole Rh ↑	+ 114	7 r
3 D whole Rh ↑	+ 111	
		unpolarised 1 r
4 D whole Rh ↑	+ 63	5 r
		unpolarised 2 r
6 D whole Rh ↑	+ 28	3 r
1 D whole Rh ↓	+ 80	− 26
2 D whole Rh ↓		− 19
4 D whole Rh ↓	+ 15	− 14
6 D whole Rh ↓		− 12
9 D whole Rh ↓		0
1 D whole Rh ↓		− 22

Distance E_1 E_2 12 mm.
E_2 E_3 3 „
E_3 E_4 26 „

2 r means two divisions of the scale to the right.

↑ in the nerve deflects to right.

In Experiments 30, 31 and 32 the stimulating electrodes were in the middle of the intrapolar area. Two pairs of nerves in reverse order were used in each experiment in order to have a uniform cross section; and in this case the direction of the current is reckoned with reference to the nerve whose central end is next E_1.

As is shewn very well in Experiment 30, the intrapolar effect and the extrapolar anodic effect rise and fall together. The maxima and minima of the two effects correspond closely enough. Evidently the circumstances which favour the intrapolar, favour also the extrapolar phenomenon. This is very different from the case of extrapolar stimulation. There the extrapolar anodic variation was at its height, when the intrapolar variation had vanished, and it was positive in sign. Here there is no hint of a positive variation. Again we are forced to the conclusion that the decrease of conductivity about the anode, or in the whole intrapolar area, is the cause of the positive effect with extrapolar stimulation, the cause of the failure of all effect with intrapolar stimulation.

When the cathodic region is led off, the stimulation effect is always insignificant. In Experiment 31 there was a positive deflection of 19 on stimulating the unpolarised nerve. This was never reversed, but only diminished. There are two ways in which the diminution may be explained. We know that the conductivity is soon diminished round the cathode; and the smaller positive deflection might be the ordinary action current weakened to correspond with the weakened excitation. But there is another explanation. The negative variation of the cathodic electrotonic current might be strong enough only to diminish the original action current, but not strong enough to annul or reverse it. In Experiment 33 a small increase of the original action current seems to be got. For the weaker currents this might be explained as due to an increase in excitability at the point of stimulation. It may, however, be that the positive intrapolar effect, acting as an increase of the polarising current for the moment, does cause a small increase in the electrotonic currents, which is masked on the anodic side by the large negative variation, but appears on the side of the cathode. It would not be difficult to settle this by special experiments. For the present, however, I am obliged to leave it open.

Before we come to the experiments after opening the polarising current, it will be well to discuss briefly the results up to this point. And at the outset it should be remembered that a deflection obtained by stimulating a nerve may be the expression of more than one process,

the algebraic sum, it may be, of several terms. In the above experiments we had to take account first of the ordinary negative variation. Except in special cases, the nerve was arranged so that this should be small and negligible. If the negative variation is large, it is not possible, as a rule, to reckon up the share which it takes in the compound deflection. A mere process of addition or subtraction would land us in error. For example, if we had a large negative variation in the intrapolar area, this, as well as the ordinary "change of resistance" effect, would disappear when the excitation had to pass the cathodic block. If, in these circumstances, we were to correct our zero reading by the amount of the previously determined negative variation, it is plain that one of the most important facts would be obscured, namely the cathodic block itself. I know of no way of avoiding the difficulty, except by making the negative variation small.

In the next place there may be currents of action peculiar to the polarised nerve, which disappear with the polarisation. If at any two points of the polarised nerve which are connected to the galvanometer the excitation arrives with unequal intensity, an action current (using the words in the sense already explained) will be set up between them. In the intrapolar region, for example, when the block at the cathode is established, there will certainly be an action current in the same direction as the polarising stream, when the excitation comes in over the anode. Nothing seems more sure than that "polarisation increment," in this sense, must play a part in the intrapolar effect.

In the extrapolar region also, when one of the galvanometer electrodes is common to the polarising circuit or very close to it, there must be an action stream between the two points led off, if an alteration of the conductivity of the nerve is caused by the current, and if this alteration is chiefly confined to the intrapolar area and its immediate neighbourhood. I propose to call an action current conditioned by a modification of conductivity which is not permanent and therefore not due to injury, a secondary action current. These currents may be small or they may be large, but they must arise, and must take part in the stimulation deflection.

Finally a true electrotonic variation in Bernstein's and Hermann's sense may be present, and this both in the extrapolar and intrapolar regions.

There is ample evidence, as Hermann shewed long ago, that the conductivity is reduced around the cathode even with comparatively weak currents, and that the condition deepens into utter impassability

when the current is increased. There is no such direct evidence of an increase of conductivity around the anode. But there is direct evidence that the conductivity is greater around the anode than around the cathode, at least for moderately strong streams, and this is all that is required to condition a positive secondary action current in the intrapolar area. So long as there is a difference in favour of the anode, it matters not whether its absolute conductivity is above or below the normal. So far then as the direction of the intrapolar effect is concerned, it may be entirely a secondary action current. Its quantitative relations are also explained, at least above the point where the cathodic depression begins to shew itself. For its maximum, with extrapolar stimulation on the anodic side and for intrapolar stimulation, is reached with something like the current density which gives full cathodic block. Since the conductivity around the anode is continuously diminishing after this, the difference between it and zero (the cathodic conductivity) can never be greater.

The direction of the negative extrapolar effect on the side of the cathode can be explained in the same way. But, if the negative variation on the anodic side is an action current, it is necessary to assume that the conductivity of points nearer the anode is greater than that of points farther away, that is, practically Hermann's law of polarisation increment. This is, indeed, a legitimate assumption, but as I have said, there seems to be no direct evidence in its favour. A change of polarisation constant is, therefore, equally in court. But as the current is increased, the conductivity diminishes around the anode and in the intrapolar region generally. On any hypothesis the time will come, when an extrapolar point remote from the anode will have a greater conductivity than the anode itself or a point close to it. This is the necessary condition for a positive secondary action current in the anodic area. This, if we suppose the direction of the electrotonic variation to be unaffected, will come into conflict with the latter, and it is the difference of the two which the galvanometer will shew. When there is complete block at the anode the difference of conductivity between the two points will be as great as it can be, unless we assume that the conductivity may increase at the point which is more remote. This is the condition, then, which will determine the maximum of the positive secondary action current. But if $u = x + y$, u may be negative if x and y are both negative; or if x is positive and y negative, but y greater than x. Again u may be positive if x and y are both positive; or if x is positive and y negative, but x greater than y. In our case u,

the observed deflection, is negative with the weaker currents, positive with the stronger; and we have to consider the reason of the change of sign. Whatever the explanation of the negative electrotonic variation may be, I do not think that it is mainly a secondary action current. It occurs when the led off area is far from the polarising circuit, and with weak currents. Hermann has, indeed, supposed that his law of polarisation increment was necessary to explain the difference in amount between the anodic and cathodic variations; but he does not now maintain that this is the essence of the phenomenon.

We have seen that even when the galvanometer and battery circuits touch each other by a common electrode, the variation on the side of the anode is still negative with the weaker currents. On the side of the cathode the variation is always negative, but it seems to be less when the led off area is very near the battery circuit than when it is farther away (Experiments 21 and 22). This is the opposite of what happens with the positive anodic variation. Now the secondary action current is negative on the cathodic side, and must necessarily be greater when the two circuits are close together. The idea arises that there may be a positive variation which opposes this action current, and keeps it down, although it is not strong enough to reverse it.

On the anodic side, as the pole becomes impassable, the negative variation changes into a positive variation, which is generally much larger than the cathodic effect. The secondary action current is here positive, and, if there is a positive electrotonic variation, it would add itself to this. What reason is there for supposing that a true positive electrotonic effect is developed?

On the theory that stimulation produces a change in the capacity for polarisation it is not difficult to see how an excitation which only affects the extrapolar region, and especially an excitation which fails to reach the part of this region next the polarising circuit, would cause a strengthening of the electrotonic twig[1]. Now, under the conditions of our experiments, this was nearly always the state of affairs on the side of the cathode, and the same is true of the anodic side for currents which gave the positive variation. A positive electrotonic variation is, therefore, what we might expect.

But there is some more direct evidence. The after-effect is positive on the anodic side, negative on the cathodic, that is it has the same sign as the stimulation effect during the flow. If the after-effect is due to a secondary action current, the changes of conductivity which cause such

[1] See Hermann, Pflüger's *Archiv*. Bd. XLII. *loc. cit.*

currents during the flow must be supposed to persist for some time after opening. We shall see that there is plenty of evidence that, after opening a strong current, the conductivity is depressed around both anode and cathode, if the time of closure has been more than a fraction of a second.

In the above observations the time of closure varied from twenty seconds up to several minutes, and the conditions were, therefore, favourable for the persistence of the modifications present during the flow. The after-effects may be provisionally regarded as representing the secondary action currents. This will only be an approximation, because the effects fall away continuously with the time after opening; but they do not fall away very fast.

If now the positive anodic variation during the flow is the sum of a positive electrotonic variation and a secondary action current, the after-effect may be expected to be smaller than the combined effect. If the cathodic negative variation during the flow represents a negative secondary action current diminished by a positive electrotonic variation, the after-effect may be expected to be greater than their difference. On the whole this is the behaviour which has been observed, as is illustrated by Experiments 14 a, 14 b, 17 and 19[1]. Experiment 18, indeed, shews in one or two cases a positive after-effect a little larger than the positive variation during the flow. This may be because the conductivity was more depressed at the anode after than before the opening, as may well happen. But generally the after-effect is decisively smaller. Again the cathodic after-effect may occasionally be less than that during the flow. But this is chiefly with weak currents, and we shall see that it is only after the stronger currents that the depression of conductivity persists at the cathode. As has been pointed out, the after-effect can in any case be regarded only as an indication, in exceptional circumstances, of what happens during the flow. In order that it may indicate this aright, the conditions must be such, that the state of affairs during the flow persists for some time after opening the current. In my experience a long period of flow is the most important of these conditions, but the strength of the current is also of importance. It is only for the extrapolar regions that the conditions can be realised.

On the whole, although I do not wish to press the point, there is a good deal of evidence in favour of the existence of the positive electrotonic variation. If we take the analogy of the negative electrotonic

[1] The results cannot be due to difference of excitability at the point of stimulation during and after the flow. Any effect which this might have would rather be in the opposite direction.

variation as a guide, it is not difficult to see why the secondary action current in the cathodic area should be able to master the electrotonic variation, because the latter may be expected to be much smaller than that on the anodic side.

The conclusion I come to is that the stimulation effects during the flow of the polarising current cannot be explained entirely as secondary action currents, but are probably due to the superposition of such currents on electrotonic variations. How the latter differ from ordinary action currents it is difficult to say. The main factor in their production, at least under the conditions of my experiments, is the excitation of the led off area itself. When this is cut off from the stimulus by a block which does not prevent the spread of electrotonus, for example the cathode of the polarising current, the stimulation effect is absent. Experiments 31 and 33 are apparently against this view. But the deflections there are very slight; and even if we admit that a small effect may be caused without excitation of the actual area led off, this does not weaken the evidence which shows that such excitation is the chief factor. It is very suggestive that none of the stimulation effects, so far as my experience goes, exceeds in amount the maximum positive polarisation deflection in the intrapolar region, or the main anodic after-current in the extrapolar. They are very far from increasing indefinitely with increase of the polarising stream. Everything indicates that they are functions of a physiological rather than of a physical property of the nerve. They may, however, be functions of a physiological change in a physical property. This would explain why they should tend to a limit, but not why that limit should be the one indicated by processes which are probably purely physiological.

IV. Observations after opening the polarising current when the whole intrapolar area is led off to the galvanometer.

1. *Stimulating electrodes extrapolar.*

So far as I know the first systematic investigation of this subject was a paper published by me in this Journal, Vol. IX. p. 26 &c., of which a preliminary notice was read before the Royal Society of Edinburgh in April 1887.

It has been pointed out to me, that a year before this Mr Gotch demonstrated to the Physiological Society in Oxford[1] "the polarising

[1] This *Journal*, Vol. VII. 1886, (*Proc. of Physiol. Soc.*, Mar. 20).

effect produced on nerve by the brief passage of an electric current through it, and the diminution in this effect caused by the active state of the tissue." This of course proves that Mr Gotch knew of the direction of the intrapolar effect a year before my paper was published. Hermann, as I subsequently found, had previously observed traces not only of this effect, but also of a negative variation in the extrapolar regions, during some investigations on the stimulation effect during the flow[1].

But he had only done so casually, in the intervals between his main experiments; and, although he observes that these effects are *probably* always present after polarisation, he does not seem to have gathered any notion of their real magnitude. The explanation of them he expressly declines to discuss until he has settled the true nature of the chief phenomena of his experiments, the stimulation effects during the flow namely. So late as 1883 du Bois Reymond says "wenn der positive Polarisationsstrom während der Dauer des polarisirenden Stromes durch Tetanus des Nerven verstärkt wird, dies wahrscheinlich auch mit dem positiven Nachstrome der Fall sein wird[2]."

The special object of this part of the work was to ascertain if it made any difference in the amount of the stimulation effect, whether the excitation had to pass the anode or the cathode.

It is not necessary to enter into details as to the arrangements, as this has already been done in my previous papers. The polarising current having been opened, the nerve was thrown at once into circuit with the galvanometer; the polarisation deflections were read off; and, as soon as the image had become sufficiently steady, stimulation was made and the effect noted. Whenever a quantitative comparison was intended, the same interval was allowed between opening the polarising circuit and stimulation in every observation. In measuring the time of flow, I did not use the automatic arrangement, described in my first paper, for the shortest periods. It was all done by the watch and metronome. Irregularities are, of course, unavoidable, but they do not introduce any serious error, when spread over a whole series of observations; and what is lost in accuracy is more than made up for in convenience. In some of the experiments it was desirable that there should be no interval between the commencement of stimulation and the opening of the polarising current. Here two observations of the polarisation deflections were made, one while the nerve was at rest, the

[1] Pflüger's *Archiv.* Bd. XLII. p. 246, 1881.

[2] *Sitzungsb. der Kgl. Preuss. Akademie der Wissenschaften*, 1883, Bd. I.

other while it was being tetanised. In the latter case the stimulation effect would be added algebraically to the polarisation.

Unless it is otherwise stated, the stimulating electrodes were at the central end of the nerve. Experiments 34 to 39 are examples.

We see here that the stimulation effect may fail altogether after the opening of a strong current, if the excitation has to pass the anode; while, if it has only to pass the cathode, we may have a large effect. We interpret this as meaning that the conductivity around the anode is less than that around the cathode soon after the opening of such a current. We shall see later on that this is especially true, when the time of flow is short. Even where the effect appears after descending current, it is in general less than after ascending. Occasionally after ↓ the sign of the effect is positive.

EXPERIMENT 34.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.	Very short time of flow means not more than ¼".
7 D ↓	very short	− 70 + 85	− 30	Stimulus 100
10 D ↓	"	− 110 + 190	0	" 200 (this stimulus was quite effectual before)
" ↑	"	− 75 + out of field	− 15	Stimulus 200
" ↓	"	− 150 + 195	+ 10	
" ↑			In 1′ 0 − 35	

EXPERIMENT 35. Here the polarisation deflections were taken first while the nerve was at rest, and again while it was being tetanised.

Polarising current.	Time.	Tetanus.	Rest.	
10 D ↓	very short	− 265 + 270	− 170 + 260	Stimulus 100
10 D ↑	"	− 100 + 310	− 95 + 426	"
10 D ↑	"	− 110 + 335	− 90 + 365	
10 D ↓	"	− 370 + 270	− 190 + 275	
10 D ↑	"	− 110 + 348	− 100 + 395	

EXPERIMENT 36.

Polarising current.	Time.	Stimulation effect.	
10 D ↓	very short	0	Stimulus 100
10 D ↓	"	0	"
10 D ↑	"	− 15	1′ after opening polarising current
10 D ↑	"	− 25	

EXPERIMENT 37. Short time of flow means not more than ½″ nor less than ¼″.

Polarising current.	Time.	Stimulation effect.	
6 D ↓	Short	− 30	Stimulus 140
6 D ↑	"	− 185	"
6 D ↑	5″	− 48	In 2′ after opening current
6 D ↓	5″	0	Tendency to move in positive direction
		In 1′ − 30	
10 D ↓	1″	0	
		In 1′ − 5	
10 D ↑	1″	− 45	

EXPERIMENT 38.

Polarising current.	Time	Stimulation effect.	
			Stimulus 140
12 D ↓	very short	+ 10	In 1′
		+ 2	In 2′
12 D ↑	"	− 84	In 30″
		− 45	2′
		− 25	3′
		− 18	4′
		− 10	5½′
		− 7	7′
		− 5	9′
12 D ↓	5″	+ 10	In 1′
		+ 3	2′
		+ 5	4′
		+ 6	9′

EXPERIMENT 39. Nerve was crushed over lower polarising electrode. There is now a strong ascending current in nerve, and on stimulation 180 divisions of negative variation.

Polarising current.	Time.	Stimulation effect.	
12 D ↓	short	+ 10	In 30″ after opening.
		+ 45	1½′ "
		+ 165	3½′ "
		+ 175	5′ "
12 D ↑	short	− 210	1′ after opening.
		− 185	3′ "
		− 153	4′ "
		− 190	7′ "
		− 192	9′ "
			Repeated crushing. Negative variation now 207.
12 D ↑	short	− 201	In 30″ after opening.
		− 195	1½′ "
		− 198	3′ "
12 D ↓	short	+ 15	30″ "
		+ 80	1½′ "
		+ 205	3′ "
		+ 204	4′ "

Experiment 24 is very instructive and it will be well to discuss it briefly here leaving the others to be dealt with later on. It is plain that after the descending current the positive deflection got on stimulation represents the negative variation in the ordinary sense; and we may suppose that the excitation is blocked at the anode, and that the block gradually gives way. We know from the other experiments in this group that there is no negative effect after a strong descending current, so that we cannot suppose that the smallness of the positive deflection here in the first minute or two after opening the current is due to an opposing negative effect. The positive deflection is therefore in this case a measure of the conductivity of the nerve around the anode. And it never attains a higher value than the original negative variation. But we might suppose that in the case of the ascending current we ought to get the original negative variation added to the negative deflection caused by stimulating the polarised nerve. The apparent negative stimulation effect ought then to be very much larger than the original negative variation. We see, however, that it is only a little larger after the first crushing, and actually somewhat

smaller after the second. The reason is no doubt this. The excitation, passing the region of the cathode freely, is blocked in the uninjured nerve as it nears the anode after the opening of a strong current. It is effectually blocked; but it cannot be more effectually blocked than it is by the crushing of the injured nerve. In other words in the nerve which has been crushed over the anode the nerve impulse and the negativity which is bound up with it develope a certain strength upon the cathode, but fall away to nothing on the anode. Again in the uninjured strongly polarised nerve they develope on the cathode a certain strength not differing probably very much from that developed in the unpolarised nerve; and on the anode this also as we have seen falls away to nothing. Accordingly the difference of potential between cathode and anode will be pretty nearly the same in both cases, and therefore the deflection produced by stimulation will be nearly the same.

2. *Stimulating electrodes intrapolar.*

The circuit of the secondary coil was only closed after the opening of the polarising current.

EXPERIMENT 40.

Polarising current.	Time of flow.	Polarisation deflections.	Stimulation effect.	
2 D ↑	5″	− 125	− 12	Two nerves in reverse order.
2 D ↓	5″	− 151	− 7	Distance between polarising electrodes 26 mm.
5 D ↑	1″	− 108 + 24	− 9	Stimulating electrodes in middle of intrapolar region.
5 D ↓	1″	− 136 + 35	− 10	
5 D ↑	5″	− 375	− 14	
5 D ↓	5″	− 345	− 13	
12 D ↓	short	− 201 + 190	− 31	
12 D ↑	short	—	—	
10 D ↓	5″	− ∞	− 25	
10 D ↑	5″	− ∞	− 44	
7 D ↓	½″	− 140 + 52	− 19	
7 D ↑	½″	− 102 + 68	− 14	

EXPERIMENT 41.

Polarising current.	Time of flow.	Polarisation deflections.	Stimulation effect.	
5 D ↓	½″	− 70 + 63	− 7	Only part of nerve below branches for thigh used.
5 D ↑	½″	− 77 + 32	− 5	Distance between polarising electrodes 16 mm.
8 D ↑	½″	− 111 + 92	− 15	Stimulating electrodes in middle of intrapolar region.
8 D ↓	½″	− 102 + 140	− 26	
12 D ↓	½″	− 137 + 233	− 31	
12 D ↑	½″	− 140 + 305	− 35	
12 D ↓	½″	− 137 + 272	− 37	
5 D ↑	15″	− ∞	− 9	
5 D ↓	15″	− ∞	− 14	
5 D ↑	15″	− ∞	− 12	
5 D ↓	15″	− ∞	− 10	

Here it will be seen that, on the whole, the direction of the polarising current is without influence on the amount of the stimulation deflection. Small differences there are, but they eliminate each other; for sometimes after the ascending current the stimulation effect is a few divisions more than after the corresponding descending current, sometimes it is the opposite which happens. These small irregularities are no doubt partly due to the difficulty of regulating the time of closure, when it is short, by the metronome. There is no reason why with symmetrically placed electrodes, uniform cross section of the nerve, and median stimulation, the stimulation effect should depend on the direction of the current, unless there are differences of excitability and conductivity to begin with in different parts of the piece of nerve used. If there are such differences, if, for example, the nerve is crushed or seared over one of the polarising electrodes, then we should expect from the previously quoted results, to find that when this electrode is the anode the stimulation effect should be less than when the other electrode is the anode. For we have seen that, at least for short closure time, it is around the anode that the conductivity is most lowered after the opening of the polarising current, and the conductivity of the crushed part of the nerve cannot, of course, be affected. Experiment 42 shews what happens here.

EXPERIMENT 42. Nerve crushed over lower polarising electrode.

Polarising current.	Time of flow.	Polarisation deflections.	Stimulation effect.
18 D ↓	very short	− 255 + 240	− 19
18 D ↑	"	− 197 + 202	− 7
18 D ↓	"	− 152 + 249	− 17
18 D ↑	"	− 189 + 170	− 8

The stimulation effect, it will be observed, is rather more than twice as great with ↓ as with ↑; that is to say, it is important that the anode should be on an uninjured part, if a large effect is to be got. A short time of flow was used here, because with a long time of flow we know that the conductivity around the cathode remains depressed, and therefore the difference could hardly be expected to be so great.

V. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT, WHERE AN EXTRAPOLAR REGION IS LED OFF TO THE GALVANOMETER.

1. *Stimulating electrodes extrapolar.*

The stimulating electrodes were as usual at the central end of the nerve. In Experiments 43—49 there was an electrode common to the galvanometer and polarising circuits. The sign of the stimulation effect is reckoned as before with reference to the direction of the polarising current in the nerve.

When the polarising current is ascending, we get a cathodic after-current in the same direction; when the polarising current is descending, the main anodic after-current in the opposite direction is the one which affects the galvanometer. (The preliminary kick in the direction of the polarising current, which Hermann has investigated, was seen; but it is very transitory.)

EXPERIMENT 43.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↓	very short	+ 25	Stimulus 200.
6 D ↑	"	− 10	"
6 D ↓	"	+ 17	"
6 D ↑	"	0	"
6 D ↓	"	+ 23	"
12 D ↑	"	− 5	"
12 D ↓	"	+ 6	"
		+ 38	Stimulus 140.
		+ 8	In 2′.
12 D ↑	very short	− 11	Stimulus 140.
12 D ↓	"	+ 16	"

EXPERIMENT 44.

Polarising current.	Time of flow.	Tetanus.	Rest.	
6 D ↓	very short	− 186		
6 D ↓	"		+ 7 − 201	
6 D ↑	"	+ 15	+ 12	
6 D ↑	1″	+ 40	+ 28	
6 D ↓		+ 30 − 150	+ 10 − 180	
				Stimulation in 30″ gives + 25.
6 D ↓		+ 40		Stimulus 140.
6 D ↑		0		"
6 D ↓	1′	+ 55		"

EXPERIMENT 45.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↑	2″	+ 10	Stimulus 140.
6 D ↓	2″	+ 55	"
		+ 20	In 1′.
6 D ↑		0	
6 D ↓	30″	+ 37	
		+ 35	In 1′.
		0	In 3′.
6 D ↓	10″	+ 72	
		+ 7	In 2′.

EXPERIMENT 46.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↓	10″	+ 48	Stimulus 140.
1 D ↓	30″	+ 4	,,
1 D ↑	30″	+ 3	,,
3 D ↓	30″	+ 7	,,
6 D ↑	30″	− 15	,,

EXPERIMENT 47. Stimulation at *distal* end of nerve. Nerve divided from muscle.

Polarising current.	Time of flow.	Stimulation deflection.	
6 D ↑	1″	+ 30	Stimulus 140.
6 D ↓	1″	− 11	,,
6 D ↑	10″	+ 20	,,
6 D ↓	10″	− 20	,,

EXPERIMENT 48.

Polarising current.	Time of flow.	Stimulation deflection.		
1 D ↓	30″	− 3		Stimulus 400.
3 D ↓	30″	+ 21		,,
6 D ↓	30″	0		,,
		+ 7	In 1′.	,,
		+ 45	In 2′.	Stimulus 140.
6 D ↓	5″	+ 90		,,
6 D ↑	5″	− 25		,,
6 D ↓	5″	+ 80		,,
6 D ↑	5″	− 34		,,
6 D ↓	5″	+ 72		,,
		+ 16	In 1′.	,,
		+ 4	In 3′.	,,
3 D ↑	5″	+ 3		,,
6 D ↑	30″	− 58		,,
1 D ↓	5″	+ 2		,,
1 D ↓	30″	+ 8		,,
1 D ↓	3′	+ 28		,,
		− 20	In 30″.	,,
		− 70	In 2′.	,,
		− 40	In 5′.	,,
6 D ↓	10″	+ 65		,,
		+ 40	In 1′.	,,
		+ 30?	In 2′.	,,
1 D ↓	3′	+ 27		,,
		+ 11	In 1′.	,,
		+ 2	In 2′.	,,

After the descending current, except in Experiment 47, the stimulation deflection is always in the same direction as the polarising current, or in the opposite direction to the main after-current. After the ascending stream the effect is, in the first place, in general far smaller than after the descending; secondly it is usually, with short time of flow, of negative sign with respect to the direction of the polarising current, i.e. in the opposite direction to the cathodic after current. With longer time of flow, even with comparatively weak streams, it becomes positive and therefore has the same direction as the cathodic after-current. With a pretty strong stream, and a closure of several seconds, the positive effect after the ascending current may assume such proportions as to be of the same order as the effect after the descending current. Experiment 48 illustrates this.

Experiment 47 shews that it is not a difference of ascending and descending current with which we have to deal. For, when the stimulating electrodes are placed at the distal end of the nerve, everything is reversed. It is now after the ascending current that the large positive effect is got; after the descending current the effect is negative.

Experiment 49 shews that with the long time of flow (2′) the effect on the cathodic side may equal that on the anodic. The great tenacity of the modification on which the effect depends is well brought out. As to the extrapolar polarisation currents, although it is usually stated that the cathodic current disappears very quickly, I am convinced that this is only true if the time of closure be short. With a time of closure of 1′ and upwards and a fairly strong polarising stream, the cathodic current seems to hold its ground even more stiffly than the anodic.

EXPERIMENT 49.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
5 D ↑	very short		− 2
5 D ↓	„		+ 11
5 D ↓	5″	− 148	+ 19
5 D ↑	5″	+ 45	− 9
5 D ↓	5″	− 154	+ 22
5 D ↓	1′	− 182	+ 36
	in 1′ after opening		+ 25
	„ 2′ „	− 10	+ 26
	„ 3′ „		+ 18
	„ 8′ „		0
5 D ↑	1′	+ 162	− 28
	in 1′ after opening	+ 125	− 26
	„ 2′ „	+ 115	− 21
	„ 3′ „	+ 105	− 15
	„ 4′ „	+ 96	− 11
	„ 6′ „	+ 81	− 4
	„ 8′ „	+ 70	− 3
5 D ↓	2′	+ 51 / − 154	+ 30
	in 1′ after opening	− 35	+ 27
	„ 2′ „	+ 8	+ 21
	„ 3′ „	+ 31	+ 15
	„ 4′ „	+ 52	+ 10
	„ 5′ „	+ 75	+ 4
	„ 7′ „	+ 99	− 2
	„ 9′ „	+ 121	− 6
	„ 11′ „	+ 120	− 7
	„ 15′ „	+ 141	− 11
5 D ↑	2′	− 245	− 31
	in 1′ after opening	− 215	− 29
	„ 2′ „	− 199	− 28
	„ 4′ „	− 188	− 23
	„ 6′ „	− 169	− 16
	„ 10′ „	− 141	− 4
	„ 12′ „	− 124	− 1
	„ 16′ „	− 103	− 4
	„ 18′ „	− 93	− 6
5 D ↓	1″	− 96	+ 14
5 D ↑	1″	− 30	− 5

2. *Stimulating electrodes intrapolar.*

Here the large resistance B was in the circuit of the secondary coil in order to prevent polarisation currents from passing. This circuit was open during the flow of the polarising stream.

Experiment 50.

Polarising current.	Time.	Polarisation deflection.	Stimulation effect.
5 D ↓	1·3″	− 69	+ 17
5 D ↑	1·3″	+ 6	0
5 D ↓	1·3″	− 58	+ 19
5 D ↑	1·3″	+ 9	− 2
10 D ↓	·5″	− 102	+ 41
10 D ↑	·5″	+ 31	− 14
10 D ↓	very short	− 106	+ 34
10 D ↑	″	+ 23	− 11
5 D ↓	10″	− 126	+ 36
5 D ↑	10″	+ 40	+ 4
5 D ↓	30″	− 161	+ 46
5 D ↑	30″	+ 68	+ 2
10 D ↓	1·3″	− 95	+ 28
10 D ↑	1·3″	+ 55	− 12
10 D ↑	30″	+ 167	− 23
10 D ↓	30″	− 342	0
	in 1½′ after opening		+ 37
	in 2½′		+ 33

Distance between polarising electrodes 17 mm.
Distance between galvanometer electrodes 9 mm.
Stimulating electrodes in middle of intrapolar region.

Experiment 51.

Polarising current.	Time.	Polarisation deflection.	Stimulation effect.
Stimulating electrodes 4 mm. from lower end of intrapolar area.			
5 D ↑	1″	+ 16	0
5 D ↓	1″	− 108	+ 39
10 D ↑	1″	+ 36	− 19
1 D ↓	1″	− 202	+ 45
Stimulating electrodes in middle of intrapolar area.			
5 D ↑	1″	+ 9	− 1
5 D ↓	1″	− 104	+ 25
10 D ↑	1″	+ 21	− 7
10 D ↓	1″	− 182	+ 36
Stimulating electrodes 3 mm. from upper end of intrapolar area.			
5 D ↑	1″	+ 7	+ 1
5 D ↓	1″	− 66	+ 19
10 D ↑	1″	+ 25	− 7
10 D ↓	1″	− 180	+ 27

Distance between polarising electrodes 22 mm.
Distance between galvanometer electrodes 9 mm.

EXPERIMENT 52.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect	
		unpolarised	66	Two nerves in reverse order: crushed over upper galvanometer electrode.
10 D ↑	1″	+ 25	56	
		unpolarised	56	
5 D ↑	5″	+ 25	46	Stimulus 85.
		unpolarised	51	
5 D ↓	5″	− 118	22	
	in 3′		49	
		unpolarised	174	Stimulus increased to 50.
12 D ↓	1″	− 221	68	
	in 1′		109	
	3′		147	
	5′		155	
12 D ↑	1″	+ 41	116	
	in 1′		119	
	3′		141	
	5′		122	
12 D ↓	5″	− 360	16	
	in 1′		52	
	2′	− 72	102	
	3′		110	
	5′		143	
12 D ↑	5″	+ 134	89	
	in 1′		115	
	in 3′		123	
12 D ↑	15″	+ 280	34	
	in 45″		40	
	2′		51	
	4′		60	
	7′		67	
	12′		74	

Distance between polarising electrodes 21 mm
" " galvanometer " 12 "

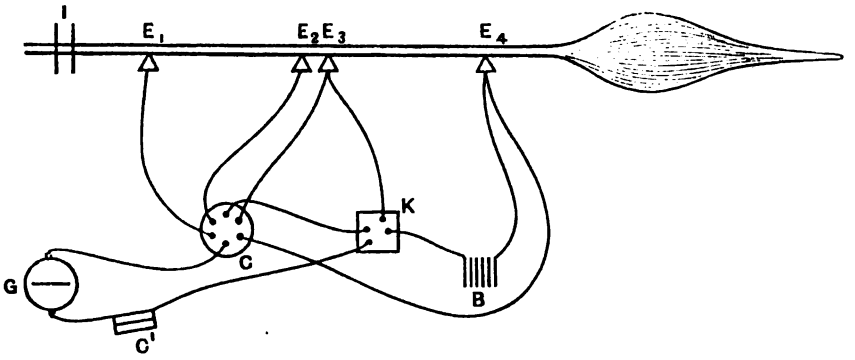
Here, as before, the sign of the effect is positive in the anodic area, negative in the cathodic. Experiments 50 and 51 show that, with the weakest currents there used and not too long a closure, there may be no effect at all on the side of the cathode. As we increase the strength of the current or the time of flow or both, the cathodic effect as well as

the anodic increases, but the former goes on growing after the latter has begun again to decline, and ultimately we may have no effect at all in the anodic area immediately after opening the current, while there is a large effect on the side of the cathode. At the end of Experiment 50, for example, 10 D ↓ closed for 30″ gave no effect at first, but after an interval a large positive deflection. The absence of effect with weak currents on the cathodic side, with strong currents on the anodic side, may be due to the same cause, or to different causes. If no modification of conductivity be left around either pole, then on our theory there may be no stimulation effect. On the other hand, if complete block be established around either pole, the effect may equally fail. I believed that the effect was wanting on the side of the cathode with the weaker currents because the modification of conductivity was slight, and that precisely the reverse was the case on the anodic side with the strong currents. Experiments, of which 52 is a type, were therefore made to determine this point. The nerve was crushed over the galvanometer electrode which was more remote from the polarising circuit. The conductivity here would therefore remain nil. The action current of the unpolarised nerve was, of course, very large, and its amount would depend upon the intensity with which the excitation passed the other galvanometer electrode, which was common to the two circuits. Changes of conductivity around this electrode would be shewn by changes in the amount of the stimulation effect, if the excitability at the stimulated point were constant. With stimulation in the middle of the intrapolar area, the difference between the action of the two poles would appear. It will be seen that the stimulation deflection was always in the same direction in the polarised and unpolarised nerve. It was always diminished just after opening the polarising current, but it was in general far more diminished after the descending than after the ascending current; and with 12 D ↓ passed for 5″ it almost disappeared. With 12 D ↑ passed for 15″ it was also very greatly diminished. This is just the behaviour we should expect, if our theory is correct. And here, as with the intrapolar effect, the amount never exceeds that of the full action stream. The action current is diminished, never reversed. That the cathode after long passage of a strong current leaves behind it a depression comparable to that left behind by the anode, we have already had abundant proofs. Some more will be furnished later on. Experiment 51 shews that the direction of the effect does not depend on the stimulation being median, although the amount may.

VI. OBSERVATIONS AFTER OPENING THE POLARISING CURRENT WHEN THE INTRA- AND EXTRAPOLAR REGIONS ARE LED OFF ALTERNATELY TO THE GALVANOMETER, THE STIMULATING ELECTRODES BEING EXTRAPOLAR.

Fig. 6 shews the arrangements in outline.

FIG. 6.



C is a Pohl's commutator without cross wires by means of which either extra- or intrapolar region could be connected with the galvanometer *G*. *K* is a double paraffin key which closed the galvanometer circuit immediately after opening the battery *B*.
C' is a compensator to balance the current of rest.

EXPERIMENT 52 a.

Polarising current.	Time of flow.	Stimulation effect.	
		Extrapolar.	Intrapolar.
5 D ↓	5″	+ 18	− 22
5 D ↓	5″	+ 30	− 22
5 D ↑	1′	− 33	− 26
	in 1′ after opening	− 18	− 39
	2′ more	− 7	− 47
	2′ „		− 31

Distance $I\,E_1$ 9 mm.
$E_1\,E_2$ 9 „
$E_2\,E_3$ 0 „
$E_3\,E_4$ 9 „

EXPERIMENT 53.

Polarising current.	Time of flow.	Stimulation effect. Extrapolar.	Intrapolar.
			unpolarised 56
5 D ↓	1′	+ 29	25
	in 1′		36
	2′	+ 13	42
	3′		47
	5′	0	58
			unpolarised 105
10 D ↓	1′		
	in 1′	+ 35	16
	2′	+ 28	25
	3′		34
	4′	+ 25	44
	6′	+ 24	73

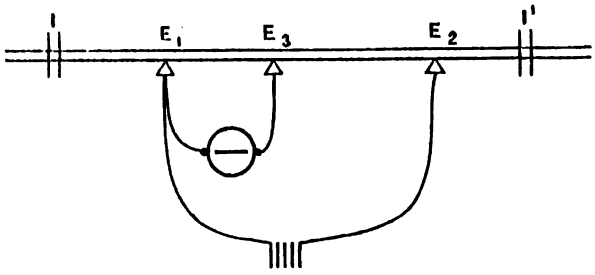
Same distances as in Experiment 52 a.
Two nerves in reverse order. Cross section on lowest polarising electrode.

I forbear from quoting more of the experiments, as the paper is already unduly lengthened. The general result is that a large extrapolar effect is associated with a small intrapolar effect, and vice versâ. Thus, in Experiment 52, after 5 D ↑ the extrapolar effect is at its height immediately after the current is opened, and falls continuously away; the intrapolar effect increases for some time after opening the current. This increase would be explained by the fact that the cathodic block does not last so long as the anodic. The former, after such a long closure time as 1′, would at first cut down the excitation greatly; later on the excitation would pass freely into the intrapolar region, but not freely over the anodic area. In Experiment 53 the full action stream in the intrapolar area is never reversed. Immediately after opening the descending current it is greatly reduced, with 10 D ↓ almost abolished. It increases continuously with the interval from the opening of the current, and regains its former amount. The extrapolar effect, as before, is at its maximum immediately after opening.

What is the cause of the stimulation effects after opening the polarising current? Are they secondary action currents, such as we assumed

in discussing the variations during the flow, or are they due to some change in the capacity of the nerve for polarisation, connected with the functional activity of the axis cylinder? It is not easy to see how the latter hypothesis could explain the results, even if we had any direct evidence in its favour. In the first place, the stimulation effect in the intrapolar area is in the direction of a diminution of the positive polarisation deflection. Assuming the existence of a true positive polarisation, we might suppose that the excitation caused a partial depolarisation. But it would be necessary to suppose further that, after the stimulation had ceased, the positive polarisation was reestablished, except so far as it would have fallen away had the nerve not been excited. For separate experiments have shewn that the positive deflection takes as long to disappear when the nerve is stimulated continuously or at intervals, as when it is left alone. Besides, everything seems to shew that this deflection is not due to galvanic polarisation. An increase in the negative polarisation would equally explain the facts, provided that this increase disappeared when the excitation was over. But how are we to imagine an increase in the negative polarisation being brought about, and how are we to reconcile

FIG. 7.



it with what takes place during the flow of the current? Again, admitting that there is an increase in the intrapolar negative polarisation, how shall we explain the decrease in the extrapolar after-currents?

If we say that the main anodic current is an action current, as Hermann has done, how shall we explain its negative variation as due to a change in polarisation? If we say that the cathodic after-current is a polarisation current, how shall we account for the fact that stimulation diminishes it, but increases the polarisation current in the intrapolar region? We turn to the explanation first suggested and ask, (1) Can the stimulation effects be accounted for as secondary action

currents? (2) Is there any evidence in favour of the existence of such currents after opening the polarising stream?

As regards the first question, if we assume that after opening the conductivity around both anode and cathode is depressed, but that the depression is greater around the former, the *direction* of the effect will be accounted for in every case. In the intrapolar area there will be a negative stimulation effect; in the extrapolar anodic area, a positive effect; in the cathodic region, a negative effect. Further, in general, the intrapolar effect will be greater when the excitation has to pass the cathode than when it has to pass the anode. The extrapolar effect will be greater, for extrapolar stimulation, on the side of the anode than on the side of the cathode; but this inequality will become less and less marked, the more the current density and time of flow are increased, because the conductivity will in both cases tend to a maximum which, we may expect, will be first reached at the anode. With median intrapolar stimulation, it is not difficult to see that at first the behaviour of the extrapolar effect will be the same; but the time will come when the relations of the two poles will be reversed, for, when the anode has become impassable, the excitation may still be able to pass the cathode. So that the first hypothesis will explain also the *quantitative* relations.

The second question as to the evidence in its favour has been partially answered in answering the first. For it is difficult to see how the facts can be explained without it. Some more direct evidence has been already got from experiments like 52, where median intrapolar stimulation was used, and the negative variation taken as the measure of the intensity with which the excitation was able to pass the poles. Further evidence will be furnished when we come to the muscle contraction experiments. The case stands thus: the phenomena can all be qualitatively explained as secondary action currents, due to differences of conductivity; their quantitative relations can also be so explained; we have proof that such action currents must arise; and finally the effects are never of a higher order than the full action stream from longitudinal surface to cross section. The conclusion does not seem doubtful.

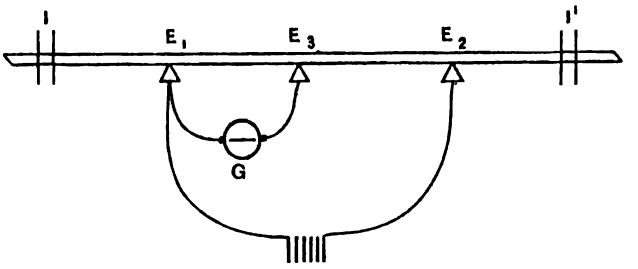
VII. Observations after opening the polarising current, when a part of the intrapolar area is led off to the galvanometer.

This section of the work was an attempt to answer the question, What is the distribution in the intrapolar region of the changes of

conductivity left behind by the polarising current? I was very desirous of answering the analogous question for the case during the flow; but I have not been able to see any satisfactory way of doing this, at least with the galvanometer, because the great resistance which would have to be put in the galvanometer circuit, to prevent short circuiting of the current through it, would cut down the stimulation effect too much. After the flow there is, of course, no such difficulty.

In Fig. 8 let E_1 be the anode and let the stimulating electrodes be

Fig. 8.



at I. If the anodic depression extends in full amount to E_3, the intrapolar galvanometer electrode; we ought to get no deflection on stimulating. If the conductivity at E_3 is unchanged, increased, or, in general, greater than that at E'_1, there ought to be a negative effect, if the excitation can pass at all. Since we must suppose that in the immediate neighbourhood of the pole, the modifications, whatever they are, which affect the conductivity are most strongly marked, we should in any case expect a negative effect; but, if the resistance of the nerve be eliminated by the introduction of sufficient resistance into the galvanometer circuit, the effect ought to be smaller when a part only of the intrapolar area is led off than when the whole is led off. If E'_1 were made the cathode, I being still the place of stimulation, a negative stimulation effect would mean that the conductivity at E_3 was less than at E_1. If this were constantly got, with every position of E_3, the inference would be that any point nearer the anode has a smaller conductivity than any point farther from the anode, that, in other words, the curve of conductivity slopes continuously down from cathode to anode. If stimulation were made at I_1, so that the excitation could not reach the galvanometer area without first passing through a portion of the intrapolar region, the problem would become more complex, because the amount of the stimulation effect would be influenced not

only by the state of the piece of nerve in the galvanometer circuit but also by that of the rest of the intrapolar region. These were the considerations which I wished to test by experiment, both for extrapolar and for intrapolar stimulation.

1. *Stimulating electrodes extrapolar.*

The important point to determine was the influence of the anode. It was arranged that a greater or less portion of the intrapolar region should be connected with the galvanometer, including either of the poles, or neither of them. In Experiment 54 the polarising electrode next the stimulating electrodes, and an intrapolar point were connected with the galvanometer. In Experiments 55 and 56 the polarising electrode most remote from the point of stimulation was so connected. The stimulating electrodes being at the central end, with descending current the anode would be led off in the former case, the cathode in the latter; but there would be this difference, that with the second arrangement the excitation would have to pass a portion of the intrapolar area, before it reached the galvanometer circuit.

EXPERIMENT 54.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
6 D ↓	5″	+ 60	0
6 D ↓	very short	+ 75	0
6 D ↑	"	− 110 + 75	− 45
6 D ↓	"	+ 70	− 4
6 D ↑	"	− 90 + 80	− 30
12 D ↑	"	− 90 + 250	− 33
12 D ↓	"	− 58 + 320	− 6
12 D ↑	"	− 95 + 250	− 36
12 D ↓	"	− 50 + 320	0

Length of nerve in polarising circuit 18 mm.
" " " galvanometer " 5 "

EXPERIMENT 55.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
Length of nerve in galvanometer circuit 10 mm.			
5 D ↓	5″	−151 + 92	−25
5 D ↑	5″	−187 + 95	−31
10 D ↑	5″	−365 +208	−37
10 D ↓	5″	−∞ +122	0
5 D ↑	5″	−194 +114	−32
Length of nerve in galvanometer circuit 7 mm.			
5 D ↑	5″	−215 +104	−30
5 D ↓	5″	−236 + ?	−27
10 D ↑	10″	−∞	−26
	in 30″		−19
	2′		−9
10 D ↓	10″	−∞	0
	in 30″		−13
	1′		−16
	2′		−8
	3′		−3
Length of nerve in polarising circuit in whole experiment 12 mm.			

EXPERIMENT 56.

Polarising current.	Time of flow.	Stimulation effect.
Galvanometer electrodes coincident with polarising.		
10 D ↑	5″	−6
10 D ↓	5″	0
Length of nerve in galvanometer circuit 11 mm.		
10 D ↑	5″	−7
Length of nerve in galvanometer circuit 6 mm.		
10 D ↑	5″	−4
10 D ↓	5″	0
Length of nerve in galvanometer circuit 3 mm.		
10 D ↑	5″	0
10 D ↓	5″	0
Galvanometer electrodes coincident with polarising.		
10 D ↑	5″	−6
Length of nerve in polarising circuit in whole experiment 12 mm. A resistance equal to about five times that of the 12 mm. of nerve was introduced into the galvanometer circuit.		

In Experiment 54 we see that, when the excitation has to pass the anode, after strong currents, there is no stimulation effect, or a small one in the usual direction. When it has to pass the cathode, the effect is large, and also in the normal direction.

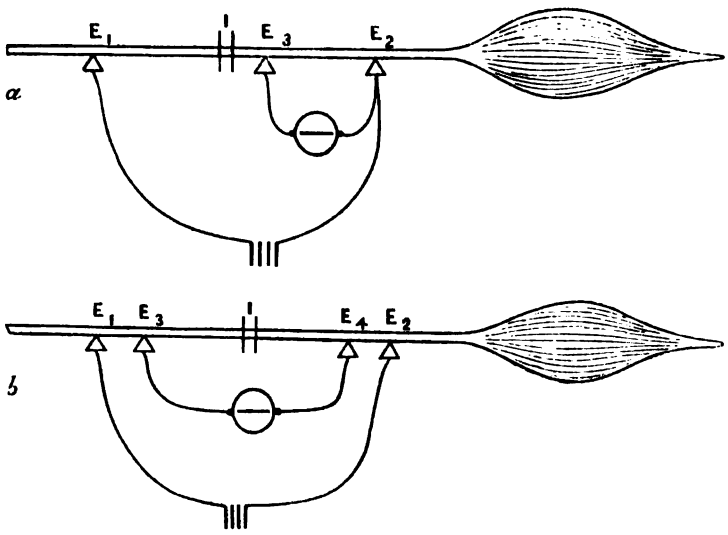
In Experiment 55 the excitation has always to pass through some of the intrapolar region, before it reaches the part led off; but the anode is a more formidable obstacle than even 5 mm. of the nerve next the cathode. Whenever there is an excitation effect at all, it is in the

negative direction as usual. Experiment 56 shews how the effect varies in amount with different lengths of nerve in the galvanometer circuit, when the total resistance in that circuit is made so great that the variation of resistance due to the nerve may be neglected. On account of the extra resistance the numbers would have to be multiplied by about 6, in order to be comparable with those of the other experiments. When the anode is next the stimulating electrodes, the effect falls out as before. When the excitation has to pass the cathode the effect diminishes, when any considerable portion of the intrapolar area lies between it and the led off part; and it disappears ultimately.

2. *Stimulating electrodes intrapolar.*

The arrangement is shewn by Fig. 9. Experiment 57 is an example of *a*, Experiment 58 of *b*.

FIG. 9.



EXPERIMENT 57.

Polarising current.	Time of flow.	Stimulation effect.
5 D ↓	·5″	0
5 D ↑	·5″	− 5
10 D ↓	·5″	− 7
10 D ↑	·5″	− 8
5 D ↑	5″	− 15
5 D ↓	5″	− 1
10 D ↓	5″	− 14
10 D ↑	5″	− 11
12 D ↑	·5″	− 36
12 D ↓	·5″	− 24

EXPERIMENT 58.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
5 D ↑	5″	− 81	− 3
18 D ↓	very short	− 82 + 121	− 11
18 D ↑	,,	− 119 + 126	− 10
10 D ↓	10″	− ∞	− 3
10 D ↑	10″	− ∞	− 3
18 D ↑	very short	− 88 + 130	− 12

Nerve crushed over polarising electrodes.

18 D ↓	very short	− 122 + 165	− 8
18 D ↑	,,	− 148 + 160	− 15
18 D ↓	,,	− 129 + 165	− 9
18 D ↑	,,	− 132 + 160	− 13

Dist. between polarising electrodes 36 mm.
Distance between each galvanometer electrode and the corresponding polarising electrode 5 mm.
Stimulating electrodes in middle of intrapolar region.
Two nerves in reverse order.

EXPERIMENT 59.

Polarising current.	Time of flow.	Polarisation deflection.	Stimulation effect.
18 D ↓	very short	− 145 + 202	− 11
18 D ↑	,,	− 161 + 145	0

The lower polarising electrode was also a galvanometer electrode, and the nerves were crushed over it.
Distance of upper galvanometer electrode from upper polarising 5 mm.
Distance between polarising electrodes 32 mm.
Two nerves in reverse order.

18 D ↑	very short	− 110 + 155	0
18 D ↓	,,	− 137 + 170	− 13

It was desirable to find whether the failure of the effect with extrapolar stimulation, when the anode was next the stimulating electrodes, was due to a block at the anode. If so, the effect would be expected to shew itself with intrapolar stimulation. Experiment 57 shews that even with the strongest currents used, the stimulation effect does not disappear after descending current. It rather increases with the strength. In general when the anode is in the led off area, the effect is greatest.

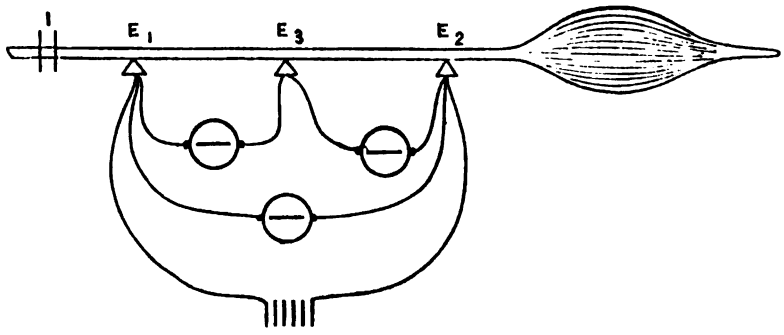
In Experiment 58, as might be expected, there is practically no difference between the two directions, at any rate before the nerves were crushed. The difference after crushing is probably due to the crushing not having been uniform over the two electrodes. In Experiment 59 when the anode corresponds to the dead piece of nerve, the stimulation effect is absent. I have never, however, under these circumstances, been able entirely to get rid of a positive polarisation deflection, when the current was strong and closed only for a short time.

Before discussing further the results of this section, it will be well to complete the account of the differential investigation of the intrapolar region, by giving some examples of experiments where different parts of the region were compared with each other.

VIII. Observations after opening the polarising current, when the whole intrapolar region and part of it or two different parts of it are led off successively to the galvanometer, the stimulating electrodes being extrapolar.

Fig. 10 is a scheme of the arrangement. Of course one galvanometer

Fig. 10.



only was required, the different regions being thrown on to it successively. The cathode was always next the point of stimulation, so as to avoid the anodic block. The electrode E_3 was exactly in the middle of the intrapolar area.

EXPERIMENT 60.

Polarising current.	Time of flow.	Polarisation deflection			Stimulation effect.		
		Anodic half.	Cathodic half.	Whole intrapolar region.	Anodic half.	Cathodic half.	Whole intrapolar region.
12 D	very short	−238 +∞	−216 +227	−∞ +∞	−12	−12	−17
5 D	"	−85 +161	−111 +31	−91 +134	−12	0	−7
5 D	"	−98 +183	−129 +36	−152 +154	−9	0	−9
3 D	5″	−242 +102	−268	−344 +45	−7.	0	−5
12 D	very short	−190 +∞	−212 +195	−∞ +∞			−14

EXPERIMENT 61.

1 D	15″	−39	−35	−51	−3	0	−4
3 D	5″	−95 +27	−148	−155	−5	−1	−3

EXPERIMENT 62.

5 D	·5″	−49 +131	−37 +32		−11	−6	
10 D	·5″	−66 +298	−92 +131		−16	−13	
5 D	5″	−169 +193	−262 +46		−21	−7	
5 D	15″	−38 +79	−350		−28	−14	
10 D	3½″	−∞ +172	−∞ +104		−39	−21 −30	
10 D	15″	−∞ +143	−∞		−33	−24	

In Experiments 60 and 61 two nerves in reverse order were used; in Experiment 62 a single nerve, but only the part below the thigh branches.

From the results of Section VIII. it will be seen, that except with

run somewhat as in Fig. 11, (2) being the effect of a moderate current, (3) of a strong, and (4) of a very strong current.

During the flow the same curves will represent the probable state of affairs, if the position of the anode and cathode be interchanged; but a much smaller current density will give a measurable effect during the flow, and therefore corresponding stages will not be associated with equal current densities. (1) represents what probably happens with the weakest currents, namely a rise of conductivity around the cathode, a diminution around the anode, while the polarising current is flowing. (1) is only partly based on my own work. Whether it would be a correct representation of the after-effects if anode and cathode were interchanged as with the stronger currents, I am unable to say.

IX. Experiments on muscular contraction with intrapolar stimulation.

1. *During the flow of the polarising current.*

In 1873[1] Hermann made experiments on muscle contraction with electrical stimulation in the intrapolar region, which included almost the whole nerve from the vertebral column to the muscle. He used only a single nerve. He found that with the descending current, the stimulating electrodes being in the upper part of the lower half of the intrapolar area, the contraction was always less than before polarisation, or might disappear altogether; with the ascending current it was always greater. He saw in this a proof of the law of polarisation increment. The fallacy which might arise from the variation of the cross section of the nerve, and the consequent variation of current density from point to point, was afterwards remarked by Hermann himself[2]. He found that, if the thin lower part of the nerve had another piece of nerve laid on it so as nearly to equalise the cross section, the above mentioned behaviour was reversed. When he used only the piece of nerve below the thigh branches, he could find no point in the lower half the stimulation of which gave his former results. Without knowing of Hermann's work at the time, I made experiments in connection with the galvanometric observations detailed above, but on different lines. All that I wished to determine was the *relative* effect of the two poles

[1] Pflüger's *Archiv*. Bd. VII., p. 323.
[2] *Ibid*. p. 497.

on the conductivity. To determine the *absolute* effect would have been very desirable, but to do this it would be necessary either to shew that changes of excitability might be neglected, or to measure their numerical amount. This I despaired of doing, and therefore confined myself to the more definite task. Some of the experiments were made with a single nerve, the intrapolar area varying in cross section. But these I decided to control and supplement by observations with approximately uniform cross section, obtaining this in some cases by using only the pieces of the sciatic below the thigh branches, but more frequently by laying two nerves of the same frog together in reverse order, both muscles being left attached. In the latter case the cross section would not be perfectly uniform, but would be nearly so. When the two preparations were found to have a nearly equal excitability, which unfortunately did not generally happen, the contractions of the two muscles caused by the same stimulus were simultaneously registered. This was probably the most accurate method of all, because there could be no question of differences of excitability. In order to eliminate these when two successive observations had to be taken with the current flowing in different directions, the stimulating electrodes were placed exactly in the middle of the intrapolar area[1]. Four narrow transverse grooves were made in a small slab of paraffin, two for the stimulating electrodes and two for the polarising. The grooves were filled with clay, and between them the paraffin was hollowed out, so that the whole formed a platform on which the nerves lay without touching it except at the grooves. The two central grooves were very close together and each was separated by the same interval from the corresponding groove of the outer pair. The non-polarisable electrodes were connected with the grooves. A rather more convenient arrangement, which allowed the distance between the polarising electrodes and the position of the stimulating pair to be easily varied, was got by fixing a paper scale on a paraffin slab, to the edge of which the clay points of the electrodes carrying the nerves were adjusted. I introduced much more resistance into the secondary circuit than Hermann seems to have thought necessary, namely the large resistance *B*.

For the weaker currents the strength of stimulus was sought which was just less than that necessary to give a minimal contraction, when the cathode was next the muscle. Then a sufficient interval was allowed (in quoting the experiments this is not noted to save space), and with the current in the opposite direction, it was observed whether

[1] See former paper in this *Journal*, Vol. IX. p. 209.

contraction occurred on stimulating. For the stronger currents the stimulus was adjusted so as to give contraction when the anode was next the muscle, and the current was then reversed after an interval, and stimulation again made. The reason of the difference will appear when the experiments are looked at. It will be seen that up to a certain strength of current a stimulus will give contraction when the cathode lies next the muscle, which will give no contraction when the anode is in that position. Above this strength the reverse holds, and a stimulus which is followed by contraction when the excitation has to pass the anode, evokes no response when it has to pass the cathode. The change is not very gradual, but can hardly be called abrupt. When the cathodic block first appears, it can be overcome by increasing the strength of the stimulus. It does not, however, require a great addition to the current density to make the cathode impassable for any stimulus within my limits, when once the block has become pronounced. Moreover a block which can be broken down at first may become total, if the current be left a little longer closed. With the very weakest currents I have found no difference between the poles; but the excitability of the stimulated point seems to be increased, for a stimulus which caused no contraction in the unpolarised state causes one when the current is closed, the direction of the polarising current being indifferent. For convenience we may call this the first stage, the others the second and third respectively. Hermann seems only to have met with the third stage when he used a single nerve, and stimulated in the lower half. I found the second very constant with median stimulation even with a single nerve, although not so well marked as with the double nerve; but the third stage is undoubtedly reached much earlier in this case than with uniform cross section, even where there is no extra resistance in the nerve circuit, and where, therefore, the same E. M. F. gives practically the same average density of current when the distance between the polarising electrodes is the same. But with a single nerve we must remember, that the density of the current in the thin part below the thigh branches will be several times as great as at the plexus. If we could be sure that the excitability was the same at the stimulated point for both directions of the current, this would introduce no fallacy in comparing the effect of the two poles. But in comparing experiments made on the single nerve with those made on the double nerve, it must be taken into account. Where I used the single nerve, far less of the thin part was included in the polarising

circuit than of the plexus. Accordingly, if we call the cross section at the upper electrode a, we may assume that a given E. M. F. E would give a current a little less than KaE, where K is a constant depending on length and specific resistance of nerve. Let b be the cross section at the lower electrode; then the density here would be a little less than $\frac{Ka}{b}E$. Now take the case of the double nerve. Here the cross section at the electrodes would be $a+b$, and at no part would it differ much from this. An E. M. F. E would give a current $K\ (a+b)\ E$, and the density at either electrode may be called KE. The density at the lower electrode in the case of the single nerve would therefore be nearly $\frac{a}{b}$ times that with the double nerve. I suppose it would not be above the mark to put $\frac{a}{b}=3$ in most cases. The third stage might, therefore, be expected to begin in the single nerve with about $\frac{1}{3}$ of the E. M. F. for which it first shews itself with the double nerve; any change of excitability at the point of stimulation being left out of account.

I proceed to quote a few of the experiments. Experiment 66 is an example of chemical stimulation at the middle point. A drop of strong brine was applied and left a little time, and then, before tetanus had appeared, a current of 1 D ↑ was passed. Tetanus appeared at once, and disappeared on opening the current. With the descending current there was no tetanus. With 2 D the stimulus was apparently too weak to give contraction with either direction. In Experiment 67 mechanical stimulation was used, also at the middle point.

Experiment 67b is an instance with marked inequality of cross section. Below 1 D Rh 900 the descending current seems more favourable than the ascending. But with 1 D and above it the opposite is the case. Experiment 67a is an example where the two muscles were observed simultaneously. As in all cases where two nerves were used, the polarising electrodes were at the same distance from the point of entrance of the nerves into their muscles, and therefore the cross section and current density at them would be equal. In some of the experiments it was noted whether contraction occurred at make or break of the polarising current or at both. The second stage as a rule passed into the third with a current which was either Pflüger's "strong" current or a strong "medium."

EXPERIMENT 63.

Polarising current.	Muscle contraction on stimulation.	Stimulus.	Muscle contraction on making or breaking pol. current.
1 D Rh 1000 ↓	No	100	
↑	C	,,	
,,			
2 D ↓	No	90	C at M & B.
2 D ↑	C	,,	C at M.
3 D ↓	No	80	
3 D ↑	C	,,	
4 D ↓	No	,,	C at M.
4 D ↑	C	,,	C at M & B.
5 D ↓	No	,,	C at M.
5 D ↑	C	,,	C at B.
5 D ↓	No	0	
4 D ↓	No	0	
3 D ↓	No	0	
2 D ↓	No	0	
1 D ↓	large C	0	
7 D ↑	C	20	C at B.
7 D ↑	No	40	
7 D ↑	C	30	

Distance between polarising electrodes 28 mm.
Two nerves in reverse order.
C means contraction. No means none. M and B mean make and break of polarising current.

EXPERIMENT 64.

Polarising current.	Muscle contraction on stimulation.	Stimulus.	Muscle contraction on making or breaking pol. current.
1 D Rh 1000 ↓	No	80	C at M.
↑	C	,,	,,
,,			
1 D Rh 500 ↓	C	103	,,
↑	No	,,	,,
,,			
1 D Rh 1000 ↓	C	,,	,,
↑	No	,,	,,
,,			
1 D whole Rh ↓	C	,,	,,
↑	No	,,	,,
,,			
2 D whole Rh ↓	C	,,	,,
↑	No	,,	,,
,,			
3 D ↓	C	,,	,,
3 D ↑	No	,,	,,
4 D ↓	C	65	C at M & B.
4 D ↑	No	,,	,,
5 D ↓	No	48	,,
5 D ↑	C	,,	,,
6 D ↓	No	47	,,
6 D ↑	C	,,	,,

Two nerves in reverse order.
Resistance A in nerve circuit.
Distance between polarising electrodes 31 mm.

EXPERIMENT 65. Two nerves in reverse order.

Polarising current.	Muscle contraction on stimulation.	Stimulus.	Muscle contraction on making or breaking pol. current.
1 D Rh 1000 ↓	C	74	
" ↑	No	"	
1 D whole Rh ↓	C	70	C at M & B.
" ↑	No	"	C at M.
1 D ↓	C		
1 D ↑	No		
2 D (no resistance) ↓	No	66	
2 D ↑	C	"	
4 D ↓	No	50	C at M; small C at B.
4 D ↑	C	"	small C at M; C at B.

Distance between polarising electrodes 31 mm.
Resistance A in nerve circuit.

EXPERIMENT 66.

Polarising current.	Chemical stimulation.
unpolarised	No tetanus
1 D ↑	Tetanus
1 D ↓	No
1 D Rh 2000 ↑	Tetanus
" ↓	No
1 D Rh 1000 ↑	Tetanus
" ↓	Tetanus not so strong as with ↑
1 D Rh 500 ↑	Tetanus
" ↓	Tetanus not so strong as with ↑
1 D Rh 300 ↑	No
" ↓	Tetanus (strong) The tetanus is now continuous in unpolarised preparation.
1 D ↑	Tetanus increased
1 D ↓	No
2 D ↑	No
2 D ↓	No

Only a single nerve used.

EXPERIMENT 67.

Polarising current.	Mechanical stimulation (single stimuli)
1 D ↑	C
1 D ↓	C
2 D ↑	C
2 D ↓	No
3 D ↑	C
3 D ↓	No
4 D ↑	C
4 D ↓	No
6 D ↑	C
6 D ↓	No
1 D Rh 1500 ↓	C
" ↓	No

EXPERIMENT 67 a. Two nerves in reverse order.

Polarising current.	Muscle A.	Muscle B.	Stimulus.
unpolarised	C	C	45
5 D	↑ C	↓ No	"
	↓ No	↑ C	"
5 D	↓ No	↑ C	30
	↑ C	↓ No	"
5 D	↓ No	↑ C	0
	↑ C	↓ No	"
unpolarised	C	C	50

Here the excitability of the two preparations was nearly the same, and it was therefore possible to take observations on both muscles at the same time. The arrows give the direction of the current in the nerve of the muscle whose contraction is referred to.

Distance between pol. electrodes 34 mm.

Resistance A in polarising circuit.

EXPERIMENT 67 b.

Polarising current.	Mechanical stimulation (single stimuli)
1 D Rh 200 ↑	No
" ↓	C
1 D Rh 900 ↓	C
" ↑	No
1 D ↓	No
1 D ↑	C
2 D ↓	No
2 D ↑	C
3 D ↓	No
3 D ↑	C
4 D ↓	No
4 D ↑	C
5 D ↓	No
5 D ↑	C
6 D ↓	No
6 D ↑	C
7 D ↓	No
7 D ↑	C
8 D ↑	C
1 D Rh 200 ↓	C
" ↑	No

Only a single nerve. Upper polarising electrode on plexus, lower just below thigh branches.

In interpreting these results it is necessary to be careful. If we could be sure that the excitability at the point of stimulation was always the same for the two directions, or in other words that the excitation at its start had the same intensity, then it would, I think, be impossible to doubt that the impulse does alter in amount in passing along the polarised nerve. In any one experiment it is of course impossible to be sure of this, unless, as Hermann has suggested, changes of excitability in Pflüger's sense are apparent only and not real. But in a series it is highly improbable that the error should be always, or even nearly always, in the same direction. So far, indeed, as the third stage is concerned, the difference between the two directions soon becomes so marked that accidental errors due to differences of excitability may, I think, be neglected. The stimulating electrodes

may be placed far from the middle of the intrapolar region, and either above or below it, without altering the results in a qualitative way at least. On the whole I do not see how for this stage, without considering the galvanometric evidence at all, we can avoid the conclusion that the conductivity around the cathode is less than that around the anode.

I have already said that, after the cathodic conductivity has once begun to decline, the curve falls sharply till the pole is impassable for the strongest stimulation. For the anode the fall is much more gradual, and a moderate strengthening of the stimulus suffices to bring out the contraction which has just disappeared. If we increase the current now a little the contraction will again fall out, to be again restored on strengthening the stimulus, and so on. Finally the time will come, as we saw that it came in the galvanometric experiments, when even with the ascending current no excitation will pass with the strongest stimulation. But this condition will gradually blend into what preceded it. This seems to me to support the view, for which we have already seen other evidence, that only around the cathode is there a truly polar block during the flow. The gradual failure of effect with ascending current may be explained as due to a continuous diminution of excitability at the point of stimulation, or to a gradual spread of the depression of conductivity from cathode to anode along the intrapolar area, or to both. So far I think we are on safe ground. Neither is there any difficulty with the first stage. We should hardly expect to find well developed differences of conductivity with the weakest currents used. At any rate there must be a limit below which the method would not be delicate enough to detect them.

But it is more difficult to deal with the phenomena of the second stage. Here the contraction seems to be favoured by the descending current. If the explanation is to run on the same lines as before, this would imply that, for these currents, the conductivity is greater in the cathodic region than in the anodic. And there is a fact which supports this view. It was found by Rutherford and by Wundt that the velocity of the nerve impulse is increased in the cathelectrotonic, and diminished in the anelectrotonic area, when the polarising current is weak and not closed for too long a time. It cannot, of course, be assumed that the velocity of the impulse, and its intensity must necessarily be affected in the same sense. In the unpolarised nerve we do not know that the intensity of the impulse affects its velocity at all. But some conditions which diminish the latter diminish the former also,

cooling the nerve for example. And Engelmann has found[1] that in the ureter the velocity and the amplitude of the contraction wave rise and fall together, at least within certain limits, the alterations produced by a voltaic current being confined to the intrapolar area, beyond which also until a comparatively recent period galvanic electrotonic phenomena have not been observed in muscle. I am far from saying that this can be extended to the nerve impulse without further parley. But it is a hint that in nerve also an excitation which passes more quickly through a part will pass with less loss, or greater gain. v. Bezold, working with stronger currents than Rutherford and Wundt, found that the velocity of the nerve impulse is diminished both in the anodic and cathodic area. This corresponds with our third stage, if for velocity we read intensity. It would be interesting to know if the velocity, like the intensity, is more diminished around the cathode than around the anode.

The galvanometric observations in the intrapolar region give no indication of the state of matters which we have supposed to exist during the second stage. The stimulation effect is always positive even with the weakest currents. A secondary action current caused by a relatively increased cathodic conductivity would be negative. Still it is unlikely that the whole intrapolar effect during the flow is due to an action current under any circumstances, and the part of the positive effect which for want of a better name we may call the electrotonic part, might be strong enough to mask a weak secondary action current. The stage where the galvanometric effect is about equal for the two directions would then pass directly into that where the decline of cathodic conductivity diminishes the effect for stimulation on the side of the cathode. As was stated on p. 475, a positive extrapolar variation was sometimes got in the cathodic area with weak currents and especially with fresh nerves. The conditions under which it appeared are the same as those under which the velocity of the impulse is increased. If we remember that the true electrotonic variation is in the opposite direction, it will not appear surprising that this positive effect should be always small, and should only occasionally appear at all. Another point which must be considered is this, that the contraction of a muscle is a far more delicate reagent for the detection of a nerve excitation than a galvanometer deflection. Finally it ought not to be lost sight of that the nerve endings in the muscle, or even the muscle itself, may be affected by the polarising current, and this may have an influence on

[1] Pflüger's *Archiv.* Bd. III. p. 247, &c.

contraction, as well as the conductivity or excitability of the nerve trunk. There would of course be nothing corresponding to this in the galvanometric observations.

If anybody says, that there is not enough evidence for us to conclude that during the second stage the conductivity around the cathode is increased, I have no right to object. What evidence I have found has been stated, and I have tried at least as hard to find objections to the conclusions I have ventured to come to as to find arguments in their favour. The evidence shewing the relative depression of conductivity around the cathode during the third stage is very much stronger, so strong that it does not seem easy to doubt it. There is one objection, however, which ought not to be passed over. It may be said it is with comparatively strong currents that most of this work has been done; are the effects observed truly physiological, or are they due to injury of the tissue? It is a legitimate question. If the effects were due to permanent injury, the observation of them would not be without value, provided they were constant effects. In this investigation, however, I did not wish to study the behaviour of currents of injurious strength. The nerve was therefore tested as to its condition at intervals throughout every experiment. The assumption was, that a nerve which at the end of an experiment shewed something like the same behaviour qualitatively and quantitatively as at the beginning, had not been injured so far as the properties on which the particular effect depended were concerned. In a certain sense it is no doubt true that a nerve whose conductivity is completely abolished is injured; but the change is within physiological limits, if, in an isolated preparation, the original conductivity is afterwards restored.

2. *After opening the polarising current.*

For the after-effects of the current the arrangements were the same as for the effects during the flow, except that there was a break in the circuit of the secondary coil which was not closed till the polarising circuit had been opened. Further, it was not necessary to have the resistance A in the latter circuit, but the large resistance B was still kept in the former, to prevent the polarisation currents from passing. After opening the polarising stream, stimulation was at once made, and it was then repeated, sometimes at intervals of 2″, sometimes at intervals of 5″, or even at a greater interval where the block lasted a long time. Experiments 68 to 71 are examples.

EXPERIMENT 68.

Polarising current.	Time of flow.	Effect on muscle.	Stimulus.
		max. C	40
6 D ↓	5″	max. C	
6 D ↑	5″	min. C	
12 D ↓	·5″	max. C	
12 D ↑	·5″	No for 8″	
		max. C in 25″	
		max. C	38
6 D ↓	10″	max. C	
6 D ↑	10″	No for 5″	
		max. C in 15″	
		max. C	37
12 D ↓	5″	min. C at once	
		max. C in 2″	
12 D ↑	5″	No for 9″	
		max. C in 30″	
		max. C	38
12 D ↑	very short	No for 15″	
12 D ↓	,,	max. C	
		C	40
6 D ↑	very short	No for 10″	
6 D ↓	,,	C	

Two nerves in reverse order. Resistance *B* in secondary circuit.
Length of intrapolar area 31 mm.
Max. means maximal. Min. means minimal.

EXPERIMENT 69.

Polarising current.	Time of flow.	Contraction.	Stimulus.
		min. C	42
4 D ↑	10″	No for 3″	,,
		min. C	,,
4 D ↓	10″	large C	,,
		min. C	43
4 D ↓	10″	large C	,,
4 D ↑	10″	No for 18″	,,
		max. C	35
12 D ↓	15″	No for 10″	
		max. C in 5″ more	
12 D ↑	15″	No for 70″	
		max. C in 60″ more	
		max. C	33
12 D ↑	very short	No for 9″	,,
12 D ↓	,,	max. C	,,

Arrangements same as in last experiment.

EXPERIMENT 70.

Polarising current.	Time of flow.	Effect on muscle.
		max. C in unpolarised prep.
8 D ↓	5″	C
8 D ↑	5″	No for 55″
12 D ↓	1″	C
12 D ↑	1″	No for 30″
		max. in 30″ more

Only one nerve.

EXPERIMENT 70 a.

Polarising current.	Muscle A.	Muscle B.	Stimulus.
unpolarised	C	C	55
4 D 10″	↓ C	↑ No for 2″	Resistance *A* in polarising circuit.
6 D 10″	↓ C	↑ No for 4″	
6 D 1′	↓ C	↑ No for 4″	
10 D ·5″	↓ C	↑ No for 11″	
10 D 5″	↓ C	↑ No for 10″	
10 D 5″	↓ No for 5″	No for 60″	

Distance between polarising electrodes 34 mm.
The arrows give the direction of the current in the nerve belonging to the corresponding muscle.

EXPERIMENT 71.

Polarising current.	Time of flow.	Effect on muscle.
1 D ↓	20″	C
1 D ↑	20″	No for 5″
1 D ↓	20″	C
1 D ↑	20″	No for 10″

Single break shocks. Only one nerve.

The results of Section IX., 2 may be summarised as follows.

1. With a very short time of closure. *a.* If we adjust the stimulus so that contraction shall occur immediately on opening the descending current, there will be no contraction for some time after opening the ascending current. *b.* If we adjust the stimulus so that contraction shall not occur for some time after opening the descending current, it will take a longer time to appear after the ascending current.

2. With a longer time of closure, *a* always holds; but, if the stimulus be made so weak that the interval of inefficiency after the descending current is more than a few seconds, the difference between the two directions is not so marked. A strong stimulus is more suitable for bringing out the difference than a weak.

3. If we determine the strength of stimulus which is just necessary to give contraction immediately after opening, it will come out greater for the ascending than for the descending current.

4. When an interval has existed during which the stimulus has been inoperative, the contraction does not reach its original height for some time after the stimulus has again become effective; and this time is longer after the ascending than after the descending current.

The stronger the stimulus the shorter, as might be expected, is the interval between opening the current and the appearance of contraction.

Not infrequently a preliminary stage was noticed with the weaker currents, where a stimulus could be found which gave contraction at once after the ascending current but not for some time after the descending. This would correspond to the second stage during the flow, but it was very far from being as constant; and, without further investigation, it is not possible to say if such a stage really exists; or, if it does exist, how it is to be explained. With the stronger currents the above results were very constant both for the double nerve and the single nerve preparation; and also, as Experiment 70 a shews, when two muscles were observed at once.

I do not see how the conclusion can be avoided that after opening the polarising current a rapid or perhaps instantaneous reversal of the relations of the poles takes place[1]. With a short

[1] This might be explained, as Grützner has explained the break contraction, by considering the polarisation current as a new current in the opposite direction to the

time of flow the cathodic block is at once resolved on opening, and the anodic block is at once established. With a longer time of flow the conductivity is still depressed in the first moments after opening throughout the whole intrapolar region, but the maximum of the depression is found at the anode, while before the opening it was at the cathode. But what else has happened? The anode has itself become the seat of an opening excitation, probably lasting. The cathode was on closure the seat of excitation, a closing tetanus, perhaps, for the cathodic region of the nerve, if not for the muscle. Where the polar excitation reigns, there reigns also the polar inhibition which blocks the external impulse. Is there any connection between the inhibition and the excitation? It will not do to say that the nerve is exhausted by the latter, if it persists. The problem is, why does this internal excitation not break its way along the nerve? It is notorious that it does not do so except in special cases. A positive polarisation deflection is constant, a Ritter's tetanus is occasional. Is it not obvious that there is something which hems the internal excitation in? And this something, does it not also prevent the passage of the external excitation? We call the modification which does this a defect of conductivity. It is a convenient name, it is not an explanation. The relation between the conductivity of a nerve at any point and the excitability has been often discussed. It has been said that there is no necessary connection between them. It has been said that they are the same. I confess I do not see the cogency of the reasoning of that school which says that the conductivity and excitability *must* be functions of the same variable, because we cannot conceive of the nerve impulse being propagated except as an excitation of successive nerve sections by each other. Why not? Is the origin of any excitation a process like the propagation of it? I will not speak of artificial stimulation, as we see it in physiological laboratories. If I did, I might ask what analogy there is between the shattering of a nerve trunk by mechanical irritation, the electrolysis of it by electrical irritation, and the propagation of the impulse from section to section of the axis cylinder.

But I will take the case of physiological excitation of an axis cylinder process by a nerve cell, or of an end organ such as the retina by light. The two cases are of course different, but let us take the most unfavourable one. Does anybody assert that the active chemical

polarising stream, and closed as soon as the latter is opened. It would lead us, however, too far from our subject to discuss this just now.

processes in the nerve cell extend themselves to the axis cylinder process or the nerve trunk, when the impulse passes? But it will be said there is no breach of continuity; whatever processes take place in the active motor cell slide insensibly into those which take place in the active motor nerve. But where is the meeting place of the activity of the cell and the activity of the nerve, for that is the point of stimulation? Somewhere or other something which is not the nerve impulse starts the nerve impulse in the nerve. That is the point. Somewhere or other between the layer of rods and cones and the first beginnings of the axis cylinder fibrils of the optic nerve the real retinal stimulus does its work. But never again between retina and brain, between motor nerve cell and muscle, can the stimulus which a nerve section gets from its neighbour be the same as that which started the impulse. It is always now a nerve impulse which precedes a nerve impulse. I do not wish to labour this point. I do not say now that excitability and conductivity are separable properties. What I say is that we know nothing in the manner of propagation of a nerve impulse which goes against such a supposition. We know much which supports it. A good resumé of this evidence is given in a paper by E. Hirschberg[1] published in 1886. The most recent work on the subject has come from the Physiological Institute in Berlin[2]. Carbonic acid gas when applied locally to the nerve acts as if it depressed the excitability while leaving the conductivity unchanged. This was first shewn by Grunhagen. Alcohol has now been found to have the opposite effect. Gad, under whose supervision the work was done, indeed rejects the notion of separating conductivity and excitability, chiefly for the reason against which I have been arguing. He thinks the results may be explained by supposing that the nerve has what he calls a transverse irritability which is diminished by carbonic acid but not by alcohol. I do not propose to discuss the merits of this hypothesis, nor the interesting experiments on which it is grounded. These experiments leave the general question where it was. But it seems to be as well established as anything in electro-physiology that during the flow the conductivity around the cathode is diminished, at least if the current is of more than very moderate strength and that this diminution may go on to entire impassability. This was first shewn by Hermann. It has been repeatedly confirmed. Abundant evidence of its truth has presented

[1] Pflüger's *Archiv*. Bd. xxxix. p. 75.
[2] du Bois Reymond's *Archiv*, p. 350, 1889.

itself in this research. It is stated on good authority that around the cathode the excitability is increased even with strong currents. Tigerstedt found that this was true on the very pole itself with mechanical stimulation. And it is very likely that an excitation starting at the cathode will pass more easily through the remaining part of the region of depressed conductivity, than if it started with the same intensity somewhere in the intrapolar area. For Werigo found that it did not need a very strong current to block the impulse altogether for intrapolar stimulation at a little distance from the cathode. The same thing has been noticed by me in experiments where the stimulating electrodes, instead of being median, were placed at different parts of the intrapolar area. Even for the extrapolar cathodic region with descending current Engelmann[1] found that the excitability, as measured by the height of the contraction, only increased to a certain limit, as the polarising current was increased in strength, and then declined, passing on into a depression of excitability, which was more marked the nearer to the pole the point of stimulation was. When this was removed farther and farther from the pole, the depression gave place to increase of excitability. This observation, which is very difficult to explain on the ordinary view, becomes at once intelligible, if we suppose that, in addition to the cathelectrotonic increase of excitability in the whole cathodic region, there is a cathodic depression of conductivity which spreads for some distance outside the pole.

It may be said, it is not unlikely that the excitability is really diminished with strong currents near the pole. I have nothing to object. But it is certain that decrease of conductivity does not advance *pari passu* with decrease of excitability, for, if it did, the former would shew itself soonest and most decidedly in the anodic region.

To sum the matter up, there seems to be no doubt that the conductivity is depressed around the cathode during the flow except with weak currents. There is very strong evidence that the excitability is increased. What are we to say? That both things are true, and may be true at the same time. It is not difficult to think of illustrations of such a state of matters. Suppose we have a speaking tube, with a joint of thin metal at one part which can vibrate like the plate of a telephone. The more easily this is set in vibration, the more will the sound waves, set up at the mouth-piece of the tube, be weakened in passing the joint; the more will the conductivity of the tube, we may say, be diminished here. But it will be easier to set up sound waves in the tube at the joint itself, the more vibratile the latter

[1] *Pflüger's Archiv*, Bd. III. p. 403.

is. In other words the "excitability" of the arrangement at the joint will be increased as the "conductivity" is decreased. If, instead of the joint, we suppose a number of holes at one part of the tube, the same thing will happen.

Or imagine that we have two boxes of equal dimensions with transparent sides. Put into each an equal weight of metallic sodium. Heat one till it is filled with sodium vapour at a temperature below that at which it would emit light. A beam of sodium light will be weakened or, it may be, extinguished in passing through the vapour. In this box we may say the "conductivity" for the sodium light is diminished. It will require, however, less heat to make the vapour luminous in this box than to volatilise the sodium in the other, and then raise the temperature to the same point. The "impulse," that is to say, can be more easily evoked where the "conductivity" is lessened. In other words "excitability" is increased by what diminishes the conductivity.

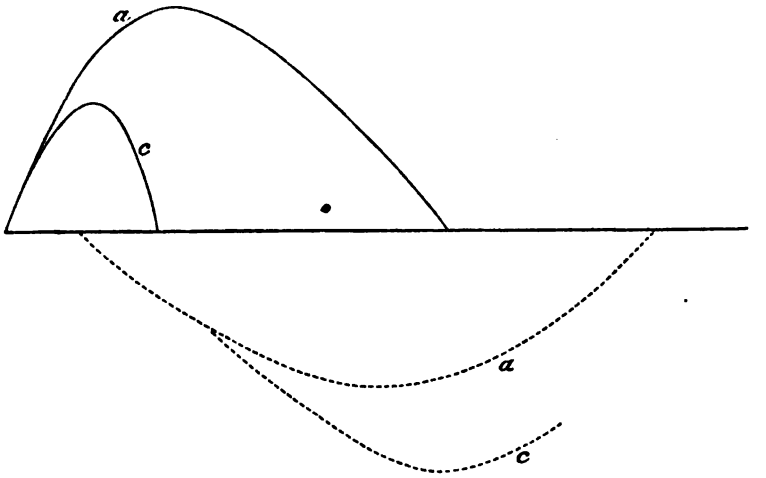
Again, to put the case a little more generally, let us suppose that the molecules of the axis cylinder transmit the nerve impulse only by vibration in the longitudinal direction. Only the component resolved in this direction of the force acting on any given molecule will be effective for the transmission of the wave. In the normal uninjured nerve we may assume that any transverse component is small, because there is no great change in the intensity of the excitation as it passes. Further, let us suppose, that in the polarised nerve vibrations in all directions are rendered easier around the cathode, the molecules being more mobile. In this region the nerve will be more easily thrown into the active state, that is, the excitability will be increased. But an impulse set up at another part will tend to waste itself in the cathodic region, by setting up those irregular vibrations which are not effective for its propagation. The conductivity will, therefore, be diminished here.

It is hardly necessary to repeat that these are only illustrations of what may happen, and are not intended to represent what does happen.

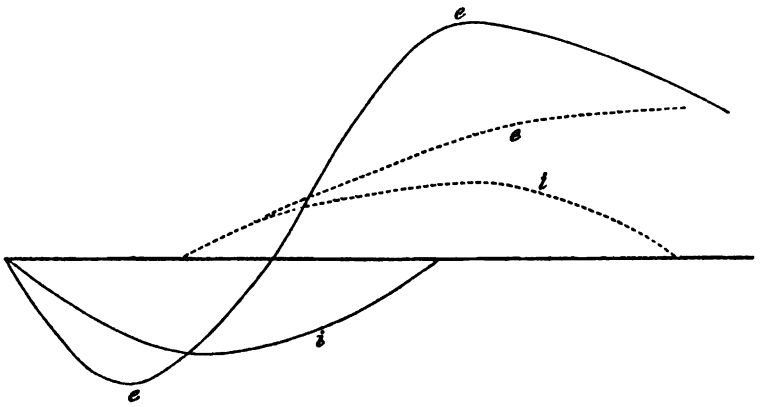
Fig. 12 shews by means of curves how the stimulation effects in the various regions are related to each other, when the current density is taken as abscissa and the galvanometer deflection as ordinate, the stimulus being supposed constant. The continuous curves represent the effect during the flow; the dotted curves, that after opening. In (2) and (3) *e* means extrapolar, *i*, intrapolar stimulation (median). In (1) stimulation is extrapolar. *a* means stimulation on the side of the anode, *c* on the side of the cathode.

FIG. 12.

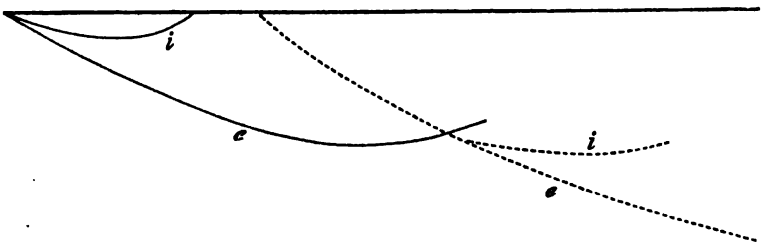
(1) Intrapolar.



(2) Extrapolar anodic.



(3) Extrapolar cathodic.



THE STROMATA OF THE RED CORPUSCLES.

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THE constituents of the mammalian red blood corpuscles are haemoglobin, lecithin, cholesterin, a small amount of proteid material, and a small amount of inorganic salts of which the most important is potassium phosphate. The blood-pigment is by far the most abundant of these constituents; about 80—90 per cent. of the dried corpuscles being composed of it. It can be dissolved out from the corpuscles by numerous reagents, such as water, ether, dilute acids, etc.; and the colourless, less soluble portion of the corpuscle which is left is called the "stroma." The stromata retain approximately the shape of the original corpuscles, but they are swollen by the reagent used to dissolve out the haemoglobin, and in the case of some reagents are ultimately dissolved.

The stromata are composed of lecithin, cholesterin, proteid, and inorganic salts. They have been the subject of research by Schmidt, Kühne, Hoppe-Seyler and Wooldridge. The present investigation was undertaken for the purpose of determining the nature of the proteid contained in them, and the plan of the research has followed very much the lines of previous experiments by one of us[1], in an investigation of the proteid constituents of the white corpuscles.

The methods which have been adopted for the preparation of the stromata are the following:—

Hoppe-Seyler's method[2]. Defibrinated blood is mixed with ten times its volume of a solution of sodium chloride (made by mixing one volume of a saturated solution of sodium chloride with nine volumes of water) and set aside; the mixture is allowed to stand for a day; the

[1] Halliburton, *Brit. Assoc. Reports*, 1887, 1888. *Roy. Soc. Proc.* Vol. XLIV. p. 255. This *Journal*, Vol. IX. p. 229.

[2] *Hoppe-Seyler's Handbuch*, 5te Aufl. 1883, p. 429.

fluid is decanted from the subsided corpuscles, which are again mixed with the salt solution, and allowed to stand another day; the process may be then repeated. This separation can be effected in a much shorter time by the use of the centrifrugal machine. The mass of corpuscles ultimately obtained is treated with water to remove the haemoglobin, then shaken with water and ether, and lastly the stromata are collected on a filter.

Kühne's method[1]. The corpuscles are obtained free from serum as in the previous case; they are dissolved in a large excess of water, and the solution so obtained is treated with a stream of carbonic acid as long as white flakes continue to separate.

Wooldridge's method[2]. Freshly whipped blood is mixed with many times its volume of 2 per cent. sodium chloride solution, and the corpuscles separated from the salted serum by the use of the centrifugal machine; the mass of corpuscles is then mixed with more salt solution of the same strength, and the process repeated several times. The corpuscles which are thus obtained free from serum are then mixed with five or six times their volume of water, and a little ether added until the solution is perfectly transparent. The leucocytes which are unaltered by this treatment sink to the bottom of the vessel, and again the separation may be hastened by the use of the centrifuge. The supernatant fluid is then treated with a few drops of a one per cent. solution of acid sodium sulphate, until the fluid, at first clear becomes as thick as the original blood. The precipitate consists of the stromata which soon collect together to form coarse flocculi, and may be collected on a filter.

We first tried Hoppe-Seyler's method; we used sheep's blood, and the corpuscles subsided so slowly without the use of the centrifugal machine that even in the winter time putrefaction had invariably commenced before the process was completed. Even with the centrifugal machine we found that the corpuscles did not at all readily settle when we used the strength of saline solution recommended by Hoppe-Seyler. We found too, that the subsequent processes did not yield an abundant supply of stromata so readily as the method we ultimately adopted. We did not try Kühne's method, as it seemed to us adapted to obtaining the globulin-like substance of the red corpuscle, rather than the whole stroma.

[1] Kühne's *Lehrbuch*, p. 193.

[2] Wooldridge, *Zur Chemie der Blutkörperchen. Du Bois Reymond's Archiv*, p. 387, 1881.

The method we ultimately adopted was Wooldridge's with one slight modification. Instead of using a two per cent. solution of sodium chloride, we employed a solution of half that strength, as we found that in it the corpuscles settled more quickly. We employed the centrifugal machine throughout: but even then the processes are so prolonged, that it took us as a rule from seven to ten days to obtain a quantity of stromata sufficient to work with.

On one point we feel inclined to differ from Wooldridge. He states that the mixture of ether and water does not dissolve the stromata, but renders them so swollen and transparent that they escape detection; the action of the acid sodium sulphate is to cause them to shrink together again.

We made several observations on this point. We took some of the watery ethereal liquid, and added it to various kinds of blood (human, sheep's, frog's) under the microscope. We found that the red corpuscles were quickly rendered colourless, and the stromata which were at first swollen, became smaller and smaller until they were invisible and apparently dissolved; the nuclei in the case of frog's blood were unaffected, and the white corpuscles in all three kinds of blood were, as Wooldridge states, apparently unaffected also.

In adding the acid sodium sulphate to precipitate the stromata we found it must be added with great care; as very slight excess renders the stromata very insoluble, and decomposes the haemoglobin. It changes the oxy-haemoglobin into methaemoglobin. By spectroscopic examination of the filtrate after separating the precipitated stromata, we usually discovered a trace of methaemoglobin; a faint band between C and D being present. This was not the band of acid-haematin. We therefore think we can safely say that the precipitate contained no globin, the proteid constituent of haemoglobin; otherwise we should have found spectroscopic evidence of haematin.

The question then arises is the whole of the stroma contained in the precipitate? Wooldridge considered that it was; as we have already seen he looked upon the action of acid sodium sulphate not as a precipitation, but as a shrinkage action upon the previously swollen stromata, and in this view of the case he is supported by his finding lecithin and cholesterin as well as proteid matter in the precipitate. If however we regard the action of the sulphate as a precipitation of something that was previously dissolved, we cannot be certain that the whole stroma is contained in the precipitate. Acid sodium sulphate is a very powerful precipitant of proteid material, as we determined in a few control experiments with diluted white of egg, and it is therefore possible that it may merely precipitate the proteid matter of the dissolved stromata in a similar way. If we adopt this view of the way in which it acts, we are no doubt confronted with the objection we just urged

against Kühne's method, namely, that ours is a method which gives us not all, but only a part of the stroma. Kühne's carbonic acid method is obviously one which would precipitate only globulin, and only part of that; Wooldridge's method is one which would at least precipitate all the proteids. We have devoted considerable attention to this objection and have arrived finally at the conclusion that, in whichever way we regard the action of acid sodium sulphate, we have in the precipitate ultimately obtained, virtually all the constituents of the stroma; even although the action of the sulphate is a mere precipitation of proteids, the precipitate carries down with it at least part of the lecithin and cholesterin; and as we were particularly concerned with the proteids, it really mattered but little to us if some of the lecithin and cholesterin remained in solution.

Having thus obtained a precipitate of the stromata upon the filter, it was quickly washed from adherent oxyhaemoglobin with distilled water containing a trace of acid sodium sulphate in solution. The operation of washing must however be rapidly performed, as prolonged exposure to water or still more to water containing acid sodium sulphate in solution, makes it very insoluble. When freshly prepared the stromata are readily soluble in 0·2 per cent. hydrochloric acid, but after a time, more and more insoluble residue remains behind after treatment with that reagent.

Our investigations of the proteids contained in the stromata consisted in making extracts of the precipitate with various saline media. In a previous research made in a similar way on lymph cells, one of us was able to distinguish in those cells the following proteids:—

1. A globulin present in small quantities only, which coagulates at the temperature of 48°—50° C. (*Cell-globulin α.*)

2. A globulin occurring in large quantities which coagulates in a 5—10 per cent. sodium chloride solution at 60°—65° C.; and in solutions containing a minimal quantity of sodium chloride, or 5 per cent. of magnesium sulphate at 75° C. This proteid (*Cell-globulin β*) is either identical with or closely associated with the fibrin-ferment.

3. An albumin which coagulates at 73° C. (Cell-albumin.)

4. A mucin-like proteid similar to that described by Miescher in pus, and called hyaline substance by Rovida. This swells up into a jelly-like substance with solutions of sodium chloride, or magnesium sulphate but not of sodium sulphate. It is insoluble in water, and is precipitated by salts like globulins. It is not mucin, as it yields no reducing sugar after treatment with dilute mineral acids. It is however rich in phosphorus, and yields nuclein on gastric digestion

in addition to albumoses and peptones. It thus belongs to the class of proteids called nucleo-albumins.

5. If the lymph cells are not examined perfectly fresh, they become acid from the development of sarcolactic acid, and the proteolytic action of pepsin, or a closely allied ferment found in the cell, comes into play with the formation of albumoses and peptone.

We may take the foregoing as giving us a list of the proteids found in typical cells, in fact the proteids of protoplasm.

The proteids of liver cells were investigated by Plósz[1] in a very similar way, and with very similar results; there are present in liver cells:—

1. A proteid coagulating at 45° C.
2. A proteid of the nature of a globulin coagulating at 75° C.
3. A nucleo-albumin.

The proteids of muscular fibres, which are elongated and somewhat modified cells, show differences from the condition of original protoplasm: the chief modification from the chemical point of view being the appearance of myosinogen, a proteid convertible by ferment action into myosin.

The red corpuscles are cellular in origin also, and the question arises, how far do they resemble, and how far do they differ from unaltered protoplasm? The presence of haemoglobin is undoubtedly something entirely different from what we find in typical cells. We must therefore look for resemblances if any exist, not in the pigment, but in the uncoloured residue, the stroma.

In view of finding an answer to this question, we made extracts of the precipitated stromata with solutions of three salts:—

1. Half saturated solution of sodium sulphate.
2. Five per cent. solution of sodium chloride.
3. Five per cent. solution of magnesium sulphate.

The first named solution (sodium sulphate) we used at first, because one of us had found it the most convenient to use in connection with the white blood corpuscles. Sodium chloride and magnesium sulphate solutions swell up the white blood corpuscles into such a slimy mass, that subsequent manipulations are greatly hindered. Sodium sulphate has not this objection. We found subsequently that sodium chloride and magnesium sulphate solutions do not cause the stromata of the red corpuscles to swell in this way, and we consequently in our later experiments used them in preference to solutions of sodium sulphate, because

[1] *Pflüger's Archiv*, Vol. VII. p. 371.

they are much more powerful solvents. We found also that the more freshly the stromata were prepared, the more readily did they dissolve in these saline media; but there was in all cases a certain amount of insoluble residue. These solutions and this residue were then examined.

It will be most convenient to describe the examination of these materials, and the results obtained therefrom, by subdividing the general question, 'How far do the stromata of the red corpuscles resemble the white in regard to their proteid constituents?' into the following five subsidiary questions.

1. Do the stromata contain cell globulin α?
2. Do the stromata contain cell globulin β?
3. Do the stromata contain cell albumin?
4. Do the stromata contain nuclein or nucleo-albumin?
5. Do the stromata contain albumoses or peptone?

1. **Do the stromata contain cell-globulin α?** We examined the temperature of heat-coagulation of the proteids in a good many saline extracts of the stromata, but there was never any appearance of a cloudiness, much less precipitate, below the temperature of 60° C. We can therefore safely say, that the stromata do not contain cell-globulin α.

2. **Do the stromata contain cell-globulin β?** This proteid will in the subsequent portions of the paper be spoken of simply as cell-globulin.

Cell-globulin as obtained from the white corpuscles resembles paraglobulin or serum-globulin very closely. Its characteristics are as follows:—

a. In solutions containing a minimal amount of salt, or from 5 to 10 per cent. of magnesium sulphate, it is coagulated at a temperature of 75° C.

b. In solutions containing 5—10 per cent. of sodium chloride, it is coagulated at a much lower temperature, 60°—65° C.

c. It is precipitable from its solutions by carbonic acid, by dialysis, and by saturation with sodium chloride incompletely; by saturation with magnesium sulphate or ammonium sulphate completely.

d. It possesses fibrinoplastic activity; i.e. it has the power of hastening the formation of fibrin in dilute salted plasma, or in pure plasma (i.e. vein plasma, or pericardial and similar fluids). It is thus either identical with, or closely connected to the fibrin ferment.

Cell-globulin resembles pure serum-globulin in characteristics *a* and *c*; they differ from one another in characteristics *b* and *d*.

From our examination of saline extracts of the stromata of the red corpuscles, we find that they contain abundance of cell-globulin. The following are the facts upon which this conclusion rests.

i. *Heat coagulation.* On heating a faintly acidified extract of the stromata made with 5 per cent. magnesium sulphate solution, it becomes opalescent at 70° C. and a flocculent precipitate occurs at temperatures varying from 73° to 77°, but most frequently at 75° C. On filtering off this precipitate, there is practically no proteid found in the filtrate.

On heating a faintly acidified extract of the stromata made with 5 per cent. sodium chloride solution, it becomes opalescent at 60° and a flocculent precipitate occurs at 65°—66° C.

On saturating a saline extract of the stromata with magnesium sulphate, a precipitate is produced. If this precipitate be washed with saturated solution of magnesium sulphate, it can be redissolved on the addition of water, the adherent salt rendering it soluble. The solution so formed is faintly opalescent; or making this solution slightly acid with 2 per cent. acetic acid, and heating it gradually, it becomes more opalescent at 70°, and a flocculent precipitate occurs at 75° C.

If the precipitate produced by saturation with magnesium sulphate be dissolved in 5 per cent. sodium chloride solution, rendered faintly acid as before and heated, opalescence occurs at 60°, and a flocculent precipitate at 66° C.

ii. *Precipitation by salts, etc.* As just stated, a precipitate is produced by saturating a saline extract of the stromata with magnesium sulphate; saturation with ammonium sulphate causes an equally dense precipitate; saturation with sodium chloride causes a considerably less dense precipitate, showing that this salt does not cause such complete precipitation of the proteid as the other two just mentioned.

A stream of carbonic acid also causes a fine precipitate.

Dialysis of a saline extract of the stromata in a stream of running water, causes the contents of the dialyser to become turbid from the partial precipitation of the proteid originally in solution. In this as in all experiments in which dialysis was employed, putrefaction was prevented by the addition of crystals of thymol.

iii. *Fibrinoplastic powers.* The globulin which is thus proved to be present in the stromata of the red corpuscles possesses marked fibrinoplastic activity. The details concerning this power may be conveniently

stated in the form of a number of propositions, after the statement of each of which, we will quote illustrative experiments.

a. A saline extract of the stromata when added to dilute salted plasma hastens its coagulation.

EXPERIMENTS. Sheep's sodium sulphate plasma was diluted in each case to six times its volume, and allowed to stand in some cases at the atmospheric temperature of about 13° C. in other cases in a water bath at the temperature of 40° C.

One specimen diluted with water; coagulation occurred

at 13° C.	at 40° C.
in 50 minutes	in 40 minutes.

Another specimen diluted with a sodium sulphate extract of stromata, which had been freed from excess of salt by a few days' dialysis; coagulation occurred

at 13° C.	at 40° C.
in 10 minutes	in 5 minutes.

Other experiments were performed with sheep's magnesium sulphate plasma, which when diluted clots more slowly than does sodium sulphate plasma.

One specimen was diluted to six times its volume with water: coagulation had not occurred 48 hours afterwards.

Another specimen diluted to the same extent with the sodium sulphate extract of stromata mentioned above, clotted in a few hours both in the water bath at 40° and in the air at 13° C.

b. The globulin precipitated from saline extracts of the stromata, by saturation with magnesium sulphate, has when redissolved, marked fibrinoplastic properties.

EXPERIMENTS. Sheep's sodium sulphate plasma was used as before, and diluted in each case to six times its volume.

One specimen diluted with water coagulated

at 13° C.	at 40° C.
in 50 minutes	in 40 minutes.

Another specimen diluted with a solution of the globulin obtained from the stromata by precipitation with magnesium sulphate, and freed from excess of salt by a few days' dialysis, coagulated

at 13° C.	at 40° C.
in 10—12 minutes	in 6—8 minutes.

c. This same globulin causes the formation of fibrin in fluids like hydrocele and pericardial fluids.

EXPERIMENTS. A quantity of ascitic fluid was divided into two parts, A and B; A underwent no further treatment and had not coagulated even after 48 hours: to B a few drops of solution of the globulin were added, and in a few hours there was a marked formation of fibrin.

An exactly similar result was obtained with hydrocele fluid, using another preparation of the stroma-globulin; while in the control specimen there was no formation of fibrin, there was after about half-an-hour (at 40° C.) a firm clot in the specimen to which a few drops of the globulin solution had been added.

d. The fibrinoplastic power of this globulin is destroyed at the same temperature as that at which the globulin itself enters into a condition of heat-coagulation. (75°—80° C.)

EXPERIMENT. A solution of the globulin obtained from the stromata by precipitation with solid magnesium sulphate was rendered faintly acid and divided into three parts A, B and C.

A was not further treated.

B was kept at a temperature of 70° C. for 15 minutes, and subsequently cooled.

C was kept at a temperature of 77°—80° C. for 15 minutes, and subsequently cooled.

Sodium sulphate plasma (sheep) was diluted to six times its volume.

	Coagulation occurred	
	at temperature of room, 14°—15° C.	at temperature of water bath, 40° C.
with water	96 minutes	53 minutes
with solution A	12 "	3 "
with solution B	12 "	3—4 "
with solution C		75 "

e. The globulin obtained from the stromata is precipitable by dialysis; on the removal of this precipitate by filtration, a good deal of globulin still remains in solution. The filtrate therefore still retains considerable but slightly diminished fibrinoplastic properties.

EXPERIMENT. A solution of the globulin was dialysed for 14 days. At the end of this time there was an abundant precipitation. The fluid with the precipitate suspended in it was divided into two parts, A and B; A was

filtered, and the filtrate used in the subsequent part of the experiment, B underwent no treatment.

Sheep's sodium sulphate plasma was diluted in each case to six times its volume.

	Coagulation occurred	
	at 13° C.	at 40° C.
Diluted with water	50 minutes	40 minutes
Diluted with filtrate from A	12 „	6—7 „
Diluted with B	10 „	4—5 „

f. When means are taken to completely separate the globulin from a saline extract of the stromata by thorough saturation with magnesium sulphate[1], all fibrinoplastic power is also removed from the solution.

EXPERIMENT. The globulin was precipitated from an extract of stromata by means of repeated saturation with magnesium sulphate, and removed by filtration. The precipitate was washed and then redissolved by the addition of water, and the solution so obtained was freed from excess of salt by dialysis. The filtrate from which the precipitate produced by magnesium sulphate had been removed was also freed from excess of salt by means of dialysis.

Sheep's sodium sulphate plasma was diluted to six times its volume with the solution of the globulin and coagulated in from 5—10 minutes. Another specimen of the same salted plasma was diluted to the same extent with the globulin-free filtrate, and had not coagulated even after 48 hours.

From all these experiments we can conclude:—

That the stromata of the red corpuscles contain a globulin that in heat-coagulation temperature, precipitability by salts and other reagents, and in ferment activity resembles the proteid called cell-globulin derived from lymph-cells or white blood corpuscles.

That therefore stroma-globulin and cell-globulin are probably identical.

The question whether the globulin and fibrin-ferment

[1] We found considerable difficulty in removing the last traces of globulin by this method; the precipitate is so fine that small quantities pass through the filter paper. However by repeated shakings with excess of the salt, and filtration many times through the same filter paper, we obtained in two specimens a final filtrate that contained no globulin and possessed no ferment activity. A similar difficulty was found by one of us in separating the last traces of globulin from the serum of horses' blood. (This *Journal*, Vol. IX. pp. 261, 262.)

are identical, or merely in close relationship with one another must still be left to a certain extent open; the balance of evidence appears to us however to be, that the fibrin-ferment is identical with cell-globulin.

Two possible objections might be urged against the last of these conclusions, namely, first that the ferment action is due to contamination with serum, and secondly that it is due to contamination with haemoglobin. In order to see whether it was possible that any constituents of serum might be present, we took a quantity of serum and treated it in exactly the same way as we had treated defibrinated blood in order to prepare the stromata, and the resulting solution gave no proteid reactions and possessed no ferment activity as tested with dilute salted plasma. This disposes of the first objection. With regard to the second, the solutions of stromata that we employed were always perfectly colourless; still in order to be quite certain, we made a solution of pure oxyhaemoglobin crystals from rat's blood, and on testing this with dilute salted plasma found that it had no power to hasten coagulation[1].

3. **Do the stromata contain cell-albumin?** We tested for the presence of cell-albumin in four different specimens of stromata. This we did by saturating saline extracts of the stromata with magnesium sulphate, filtering off the resulting precipitate and then examining the filtrate for albumin. The results obtained were as follows:—

Preparation 1. The filtrate obtained as just described contained no proteid.

Preparation 2. The filtrate gave a faint xanthoproteic reaction, but gave no precipitate on acidulating and boiling, or any other evidence of the presence of albumin.

Preparation 3. The filtrate gave a fairly well-marked xanthoproteic reaction, but no precipitate on acidulating and boiling.

Preparation 4. The filtrate gave not only a well-marked xanthoproteic reaction, but on acidulation and heating a precipitate occurred at about 70° C. This preparation however contained a small quantity of impurity in the shape of haemoglobin.

From the examination of the first three preparations which are the most trustworthy, we conclude that cell-albumin is either absent

[1] A. Schmidt originally supposed that haemoglobin was fibrinoplastic, but further researches by his pupils Rauschenbach (*Inaug. Diss.*, Dorpat, 1882) and Nauck (*Inaug. Diss.*, Dorpat, 1886), and by Wooldridge (*Practitioner*, p. 187, 1886) have shown that the power of hastening coagulation belongs not to the pigment but to the stromata.

or only present in minute traces in the stromata of the red corpuscles.

4. **Do the stromata contain nuclein or nucleo-albumin?** The red corpuscles are in mammalian animals devoid of a nucleus, and the question whether they contain the characteristic constituent (nuclein) of nuclei is not devoid of interest.

Lauder Brunton[1] was the first to demonstrate the mucin-like characters of the substance of the nuclei contained in the red corpuscles of birds, and Plósz[2] showed that this substance contains a high percentage of phosphorus, and is identical with the nuclein obtained from the nuclei of pus cells by Miescher[3].

Worm-Müller[4] appears to have been one of the first to advance the suggestion that nuclein is not confined to the nuclei but is present in the cell protoplasm also. This has been confirmed by other observers; the nuclein in the cell protoplasm is however combined with a proteid, and the compound so formed is called a nucleo-albumin[5].

Nucleo-albumin has been described in the liver cells by Plósz[6], in the cells of the submaxillary gland by Hammarsten[7], in cells from lymphatic glands and in the cells of the thymus glands by one of us[8].

In lymph cells, the nucleo-albumin[9] is the most abundant proteid present, and it is owing to this substance that the cells form a mucinoid slimy mass when treated with solutions of sodium-chloride, or magnesium sulphate.

When the stromata of red corpuscles are treated with solutions of either of these salts, there is not the faintest indication of the formation of any such slimy material: part of the stromata goes into solution, namely, the cell-globulin; part remains undissolved; the unsoluble

[1] Lauder-Brunton, *Journ. of Anat. and Physiol.*, 2nd series, Vol. III. p. 91.

[2] Plósz, *Hoppe-Seyler's Med. Chem. Unters.*, Heft iv. p. 460.

[3] Miescher, *ibid.* p. 411.

[4] Worm-Müller, *Pflüger's Archiv*, Vol. VIII. p. 190.

[5] Nucleo-albumin appears to be probably identical with the substance called plastin which has been described by Reinke and Rodewald (*Unters. aus d. botan. Lab. Univ. Göttingen*, 1881) and by E. Zacharias (*Botan. Zeitung*, p. 281, 1887). These observers have relied chiefly upon microchemical reactions for its detection in the cells. The term plastin is, however, used in a different sense by another botanical chemist, Schwartz (*Die Morph. u. chem. Zusammensetzung d. Protop.*, Breslau, 1887): he applies the name plastin to the whole of the proteid substance of cells, not to any particular constituent of it. Nuclein is apparently identical with the chromatin of microscopists.

[6] Plósz, *Pflüger's Archiv*, Vol. VII. p. 371.

[7] Hammarsten, *Zeit. physiol. Chem.*, Vol. XII. p. 163.

[8] Halliburton, *Brit. Assoc. Reports*, 1888.

[9] Nucleo-globulin would be a more correct designation.

residue is especially large if the preparation has been allowed to stand under water, or a dilute solution of acid sodium sulphate for some hours. It might be said that this insoluble residue may contain a nucleo-albumin which differs from that derived from white corpuscles, in not forming a stringy mass. It however appears to us, that the cell-globulin is practically the only proteid present, and that the insoluble residue which increases with the lapse of time after the preparation of the stromata, does not contain a different proteid, but simply the same proteid which has been rendered insoluble by the action of reagents.

The question however is not wholly settled by experiments with the stromata prepared in the way we have described; for it is possible that the reagents used may have some action upon the nucleo-albumin so that it no longer shows its characteristic properties.

Accordingly by the use of the centrifugal machine we obtained a supply of red corpuscles wholly free from serum.

These were divided into several parts; to one portion a five per cent., to another a ten per cent. solution of sodium chloride were added; to a third, fourth, and fifth portions, a five per cent. solution of magnesium sulphate, a ten per cent. solution of the same salt, and a five per cent. solution of ammonium sulphate were respectively added; but in no case was there any formation of a gelatinous mass, which when poured into water extends in cohesive strings bearing a superficial resemblance to fibrin threads. According to this experiment then, the unaltered red corpuscles do not contain the nucleo-albumin so characteristic of the white corpuscles.

The experiment was repeated twice, using other preparations of the corpuscles similarly made, with the same result.

In two other cases, however, there was a small formation, or what we took to be a small formation of this stringy substance. In one case it was fairly well marked; but in the other case one of us felt somewhat uncertain as to whether the result was to be regarded as a positive one or not. We will therefore describe the one clearly positive result we obtained.

We used defibrinated sheep's blood as in all these experiments; by centrifugalising with a one per cent. solution of sodium chloride (repeating the operation three times) we obtained the corpuscles free from serum. The volume of the mass of corpuscles so obtained was about 50 c.c.; to this we added five grammes of sodium chloride[1],

[1] This is a method we often adopted instead of adding an already prepared solution of the salt.

grinding it up with the corpuscles in a mortar till it was all dissolved. There was however no general jellying throughout the mass. On leaving this to stand for a few hours, the upper surface appeared a little clearer than the lower portions; this was skimmed off, and the lower portions of the liquid were found to be thick, but there was no coherent gelatinous appearance. The portion skimmed off from the surface was however distinctly gelatinous; on pouring it into a beaker full of distilled water, there was a formation of fibrin-like strings: these at first coloured became quite white in a few minutes and then shrank up, forming little particles floating on the surface of the water.

This specimen of blood was found on microscopic examination to contain a large excess of colourless corpuscles, and we are inclined to attribute the presence of nucleo-albumin to these, and not to the stromata of the red corpuscles.

Among previous observations as to the presence of nuclein in red corpuscles may be mentioned those of Hoppe-Seyler and Wooldridge.

Hoppe-Seyler[1] states that the nucleated red blood corpuscles of birds, reptiles, etc. contain nuclein, and a greater quantity of proteid than the non-nucleated red blood corpuscles of mammals, and that nuclein is absent from the latter.

Wooldridge[2] in one of his earliest papers states that a nuclein-like proteid (nucleo-albumin) is present in the stroma of mammalian[3] red blood corpuscles, but in so small a quantity that it was impossible to be positive; later researches however showed him that lecithin is the only phosphorus-containing substance in the stroma[4].

There thus appears to be a general consensus of opinion that nuclein and nucleo-albumin are not present in the stromata of the red blood corpuscles.

5. **Do the stromata contain albumoses or peptone?** After the separation of the proteid coagulable by heat, the saline extracts of the stromata which we prepared did not give the typical albumose reaction (a precipitate with nitric acid disappearing on heating and reappearing on cooling), nor the biuret reaction (the red colour with a trace of copper sulphate, and excess of caustic potash) so characteristic of the products of proteolysis, i.e. of albumoses and peptones.

[1] Hoppe-Seyler's *Handbuch*, 5th Aufl. p. 429.

[2] Wooldridge, *Du Bois Reymond's Archiv*, p. 392, 1881.

[3] Wooldridge does not expressly state what blood he used, but from the context we judge that it was mammalian.

[4] Wooldridge, *Practitioner*, p. 187, 1886.

On saturating a sodium sulphate extract of stromata with ammonium sulphate a precipitate was produced; this was filtered off, and the filtrate was preserved (A).

On saturating a sodium chloride extract of the stromata with ammonium sulphate a precipitate was produced; this was filtered off and the filtrate preserved (B).

On saturating a magnesium sulphate extract of the stromata with ammonium sulphate a precipitate was produced; this was filtered off and the filtrate preserved (C).

The filtrates A and B were completely free from proteid, and thus peptone (which is the only proteid not precipitable by saturation with ammonium sulphate) was absent. The filtrate C however contained proteid, but it was not peptone: the proteid present was a globulin as it was precipitable by heat, and by dialysing out the salt from the solution. This leads us to mention what one of us has confirmed by experiments with serum that the presence of magnesium sulphate partially hinders the precipitation that would otherwise be produced by saturation with ammonium sulphate. This is a somewhat important practical point in view of the large use now made of these neutral salts for the separation of proteids. It is probably explicable on the supposition that the two salts form a double sulphate ($MgSo_4 . Am_2So_4 . 6H_2O$) which has the power of precipitating proteids to a limited extent only[1].

Our experiments then have shown that albumoses and peptones are not contained in the stromata of the red corpuscles.

General conclusions. In a recent paper on the red blood corpuscles, Hoppe-Seyler[2] expresses the opinion that the chemical structure of these corpuscles is not that very generally held, that they are composed of protoplasm infiltrated with pigment, but rather that the pigment in them replaces protoplasm to a very great extent. We have instances of the replacement of protoplasm by other substances in various parts of the body: for instance, the replacement of the protoplasm of goblet cells by mucin, or of epidermal cells by keratin; and it is in a similar sense that Hoppe-Seyler appears to regard the replacement of the protoplasm of red blood corpuscles by haemoglobin or oxyhaemoglobin[3].

[1] Compare remarks on the formation of another similar sulphate in my Paper on "Proteids of Serum": this *Journal*, Vol. v. pp. 180, 181.

[2] Hoppe-Seyler, *Zeit. physiol. Chem.*, Vol. XIII. (1889).

[3] Hoppe-Seyler calls the pigment in the red corpuscles of arterial blood *arterin*, of venous blood *phlebin*. He points out that these pigments differ from the substances oxyhaemoglobin and haemoglobin that can be separated out from the corpuscles. Arterin

Our own experiments go far to support Hoppe-Seyler's views. The mammalian red blood corpuscle is not a cell in the strict morphological sense of the word; it has no nucleus. It is also not a cell in the chemical sense, for not only is nuclein absent, but the only proteid present of the four normally existing in typical cells is cell-globulin, and this exists in the stroma in small quantities only.

Previous observations on this proteid are as follows:—

Hoppe-Seyler[1] contents himself with saying that the proteid is a globulin; that it coagulates at 75° C. and is precipitable by saturation with sodium chloride.

Kühne[2] also speaks of it as a globulin; he states it is precipitable by a stream of carbonic acid; that it acts fibrinoplastically, and that it is paraglobulin.

Hammarsten[3] showed that pure paraglobulin has no fibrinoplastic action, and that A. Schmidt's name "fibrinoplastic substance" is therefore a misnomer. Fibrin is formed from fibrinogen by the activity of the fibrin-ferment, and any fibrinoplastic activity the globulin of serum may have is due to admixture with fibrin-ferment, or to adopt the nomenclature one of us has introduced, with cell-globulin, derived from the disintegration of the white blood corpuscles.

Wooldridge[4] also speaks of the proteid as paraglobulin, and states that it coagulates in a 4—5 per cent. solution of sodium chloride at about 66° C.; a fact which we have fully confirmed.

As already stated, fibrin ferment has been prepared from the stromata by Schmidt's method[5] by several of Schmidt's pupils (Nauck, Rauschenbach, Krüger).

Two observers (Krüger and Wooldridge) have found that injection of the stromata into the circulation causes extensive intravascular clotting. Krüger[6] attributes this action to the fibrin-ferment[7], Wooldridge[8] on the other hand to the lecithin the stromata contain.

is probably a compound of oxyhaemoglobin with lecithin, phlobin of haemoglobin with lecithin.

[1] *Physiol. Chemie*, p. 391. [2] *Lehrbuch der physiol. Chemie*, p. 193.

[3] *Pflüger's Arch.*, Vol. XVII. p. 447; Vol. XVIII. p. 39; Vol. XIX. p. 363; Vol. XXII. p. 431.

[4] *Du Bois Reymond's Archiv*, p. 390, 1881.

[5] That is, extracting the dried precipitate produced by alcohol, with water.

[6] Krüger, *Zeitschr. f. Biol.*, Vol. XXIV. p. 189.

[7] Birk (*Inaug. Diss. Dorpat*, 1880; Maly's *Jahresb.*, 1881), Edelberg, and Köhler have all shown that injection of fibrin ferment into the circulation generally causes intravascular clotting; but not invariably, as the normal body has the ability of destroying or resisting the action of the ferment to a very great extent.

[8] *Practitioner*, p. 187, 1886.

There have thus been two sets of previous researches; one which deals with the nature of the proteid, the other with the presence of fibrin-ferment in the stromata of the red corpuscles.

Our own work dealing with both these questions has consisted to a very great extent in confirming the results of previous observers; and in putting them together to show that the two questions are in reality one.

We have found that the proteid, though it resembles paraglobulin (or serum-globulin), is really cell-globulin: and the fibrinoplastic action of this proteid is in reality the action of fibrin-ferment, with which substance cell-globulin is probably identical.

One further question still remains to be asked. It is this;—Do the red corpuscles contribute to the formation of fibrin in coagulation as it usually occurs in shed blood? This is a question that cannot be readily answered. The red corpuscles of clotted blood are apparently normal in appearance, and do not undergo disintegrative changes, as do the white corpuscles and blood-tablets. There is therefore no necessity to suppose that the red corpuscles shed out any fibrin-ferment, at any rate, in the earlier stages of the formation of a blood clot. It is however quite possible that the ferment they contain may assist in the formation of fibrin in later stages, and thus partially account for the increase in firmness that the clot undergoes when it is allowed to remain untouched for some time. It is also possible that the existence of fibrin-ferment in the stromata may account for what Landois calls "stroma-fibrin."

This question, among others, has been taken up from the stand-point of the pathologist and therapeutist by Bonne[1]. In a pamphlet full of interesting suggestions, the probable rôle of fibrin-ferment in causing intravascular clotting in certain morbid conditions of the blood is pointed out. The normal body has the power of destroying or resisting the action of fibrin-ferment to a great extent; but in debilitated conditions this power is lessened, and the absorption of fluid exuded from wounds may produce thrombosis. An increased disintegration of white blood corpuscles within the vessels may produce a similar effect. If, however, the quantity of ferment is small, the effect is not thrombosis, but a febrile condition. Lastly, there are certain forms of disease in which the red corpuscles are destroyed within the vessels; the plasma of the blood becomes stained with haemoglobin (haemoglobinaemia); and this may pass through into the urine (haemoglobinuria or methaemoglobin-

[1] Ueber das Fibrinferment und seine Beziehungen zum Organismus von Dr G. Bonne, Würzburg (G. Hertz) 1889.

uria). In these conditions there are febrile attacks corresponding to the periods when the corpuscles are destroyed, and there appears every reason to believe that the fever is due to the presence of fibrin-ferment in the circulating blood derived from the red corpuscles. This cannot however be considered absolutely certain; we are not aware that thrombosis has ever been described in these cases, nor do any attempts appear to have been made as yet to estimate quantitatively the amount of fibrin-ferment present in the blood in this condition. The question we have investigated from the physiological point of view is thus full of interest to the pathologist also.

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ON FORMATION OF SCAR-TISSUE.

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In the course of a research by one of us (B.) into the process of occlusion of arteries after ligation, it seemed desirable to form an independent opinion of the part played by the elements of the arterial wall in the production of the fibrous tissue which eventually closes the vascular channel. The following experiments were carried out in the hope of their furnishing, to ourselves at least, some answer to the disputed question of the origin of inflammatory tissue. Are the colorless corpuscles of the blood, capable as we know them to be of passing from the vessels into the intervascular tissue, also the source of the new tissue which the inflammatory process may produce?

Cohnheim's reply to this question was an affirmative, at least as positive as are the discoveries brilliant which it was based upon. For Cohnheim the colorless corpuscles which wandered from the blood vessels in an area of inflammation were not only the source of pus cells when pus appeared, but were the formative cells for the new tissue if any new tissue were formed. From this position in the question he never withdrew. In the edition of the *Vorlesungen* only two years prior to his untimely death again with renewed insistance he supported it. It was from his laboratory that most of the observations emanated which bring evidence in favour of this "leucocytic" view. They are set forth in papers from the Institute at Breslau by Senftleben[2], and by B. Heidenhain[3]; and by Senftleben[4]

[1] The preparations with the drawings were shown in March of this year at the Royal College of Surgeons in illustration of part of the Erasmus Wilson Lectures on "Ligation in Continuity."

[2] *Virchow's Archiv*, Vol. LXXII.

[3] *Ueber d. Verfettung fremder Körper in d. Bauchhöhle*, 1872.

[4] *Virchow's Archiv*, Vol. LXXVII.

and by Tillmans[1] from the Institute at Leipsic. Among observers who independently of contact with Cohnheim published opinions in harmony with his upon this question, may be named especially Schede[2], Aufrecht[3], Bizzozero[4], and Ziegler[5].

Upon the evidence furnished by the researches of Ziegler Cohnheim laid a quite exceptional stress. The conclusions of the then Assistant in the Wurzburg laboratory have had much to do with the ascendancy of Cohnheim's teaching on the point. They have been incorporated by Ziegler himself in the well-known *Lehrbuch der Pathologische Anatomie.*

Ziegler placed little oblong chambers, made by fastening at the four corners two cover-glasses a slight distance apart, under the skin of the dog, and left them for a certain length of time. The cleft between the glasses became filled with cells, which could be examined directly under a microscope. He found these cells to be leucocytes, many of which were fatty, and resembled the ordinary cells of pus. But in many experiments after a time actual formation of tissue took place in the layer of cells between the glasses. Cells were found in all stages from the lymphoid to the epithelioid and giant-cell type. Certain cells seemed to grow large at the expense of their neighbours whose protoplasm was appropriated by the larger growing cell. He judged that his preparations proved that the giant cells of granulation tissue are at least in some cases produced from the wandering colorless corpuscles of the blood, and further that the giant cells produce both blood vessels and connective tissue. "So war dadurch der Nachweis geleistet" "dass bei der Entzündung die ausgewanderten Zellen eine gewebsbildende Rolle spielen."

Ziegler's researches were taken as furnishing experimental proof that not only do migrated white blood cells in certain numbers become pus corpuscles, but that they also in certain numbers are capable of further development, and are the primary source of cicatricial tissue.

Ziegler's conclusions were in fact similar to those of Cohnheim. A considerable number of writers have controverted them. Baum-

[1] *Virchow's Archiv*, Vol. LXXVIII.

[2] *Arch. f. klin. Chir.*, XV.

[3] *Virchow's Archiv*, Vol. XLIV.

[4] *Annali universi di Medicina*, 1868.

[5] *Exper. Unters. üb. d. Herkunft d. Tuberkelelemente*, 1875, and *Unters. üb. path. Bindegewebs u. Gefässneubildung*, 1876.

garten[1], Böttcher[2], Ewetzky[3], Weiss[4], Hamilton[5], all have at various times raised a voice against it, denying to the migrated blood cells any power of further development. According to them the formation of scar-tissue is in no way directly due to elements existing in the blood; the fibrous tissue does not arise from leucocytes. Quite notably have those histologists who have studied the process of occlusion of blood vessels assumed a sceptic attitude amid the general acquiescence in the Cohnheim-Ziegler doctrine. We will point out the writings of Thiersch[6], Riedel[7], Auerbach[8], Pick[9], Heuking and Thoma[10], and most recently and very definitely Hunter[11]; as we follow the opinions of these observers chronologically toward the present time more and more pronounced do we find the tendency to deny to leucocytes an exclusive share in the replacing of coagulum, &c. by cicatricial tissue.

From the foregoing it is evident that a very desirable side light might be thrown on the question upon which one of us (B.) was engaged, by an examination elsewhere than in a ligated blood vessel, of this capability of leucocytes to produce a fibrous connective tissue. It was determined to repeat the classical experiments of Ziegler. We may remark that we began work with an educational bias favourable to Cohnheim's view.

Methods employed.

Two circular cover-glasses, each $\frac{5}{8}$ of an in. in diameter and ·006 of an in. in thickness, were fastened together so as to form a little flat glass chamber, in the manner employed by Ziegler. A strip of tin-foil placed between them at their edge along $\frac{11}{12}$ of their circumference was cemented by shellac on each face to the corresponding surface of the cover-glass. The tiny chamber thus formed had therefore

[1] *Die sogenannt. Organis. des Thrombus*, Leipzig, 1877.
[2] Ziegler's *Beiträge zur pathologischen Anat.* II. 2.
[3] *Unters. aus der path. Instit. zu Zürich*, III.
[4] *Archiv f. klinische Chirurgie*, Bd. XXIII, 1879.
[5] Spongegrafting. *Edin. Med. Journ.*, 1881.
[6] Pitha u. Billroth. Bd. I. Abth. 2, § 549.
[7] *Zeitschr. f. Chirurg.*, Bd. VI. 1876.
[8] *Ueber die Obliteration der Arterien nach Ligatur*, 1877
[9] *Zeitschr. f. Heilkunde*, Bd. VI. 1886.
[10] *Virchow's Archiv*, Vol. CIX. 1887.
[11] Gold Medal Thesis for M.D. Edin. unpublished.

between the two ends of the strip of tin-foil an opening into the interior. The tin-foil first employed was $\frac{1}{10}$ mm. thick; that thickness was inconvenient, as the depth of the chamber was then too great for higher powers of the microscope to explore. Tin-foil $\frac{1}{20}$ mm. in thickness was subsequently employed. With this thickness membranes were obtained between the cover-glasses that made very satisfactory microscopical specimens. Fig. 18, Plate XXXIII.

These chambers for eight and forty hours before use were emptied of air and filled with distilled water previously sterilized, or with nutrient broth containing peptone according to the recipe of Koch. Both the chamber and the fluid in which it was kept were again sterilized by heat an hour or so before being used for experiment. In a few instances the air was not entirely expelled.

The animals employed by us have been in all cases rabbits or guinea-pigs. During every experiment the animal has been deeply under the influence of an anaesthetic. Antiseptic precautions were vigorously maintained throughout all the operations. No suppuration ever occurred. Had it done so in any experiment we should have excluded the results of that experiment. In our earlier experiments the chambers were placed in the peritoneal cavity; in the later into the subcutaneous connective tissue of the flank.

The chambers were allowed to remain within the animals for various periods, from four hours at shortest to 18 days at longest. When the chamber was removed its contents were examined either fresh upon a warm stage under the microscope, or after appropriate treatment with hardening and staining reagents. The outsides of the chambers were often covered with thin films of young fibrous tissue—these films were examined by the same methods as were the contents. The reagent chiefly employed was osmic acid, either in freshly made ·5°/₀ watery solution, or in vapour from a 1°/₀ solution. In the former case the chamber taken warm from the body was at once plunged into the osmic acid solution or was rapidly split open, and with the contents so exposed, placed in osmic acid. In the solution of osmic acid they remained for an hour, in the dark. Where osmium vapours were used the same plan was adopted, except that the chamber was always opened unless bubbles of air happened to have got ingress previously, as sometimes happened. Exposure to the vapour was ensured by placing the specimen between two watch-glasses, at the bottom of which was osmic acid solution, or by suspending from the cork of a bottle containing the solution. The action of the osmic acid was allowed about two hours'

play, always in the dark. The preparations with osmic acid were, after washing in water, mounted either in Farrant's gum solution, or after dehydration, in xylol-balsam. They were in some instances after-stained, with picrocarmine, with fuchsin, methylene blue, eosin, or most frequently with haematoxylin (Ehrlich's solution). Other preparations were made without the use of osmic acid. In these after hardening in chromic acid, or in alcohol, or Fleming's solution, picrocarmine, methylene blue, eosin, fuchsin, haematoxylin, etc. were used.

For the observations on living specimens a warm stage of Stricker's pattern was used.

Contents of the Chambers.

One of the first steps which we took was to examine the serous moisture of the abdominal cavity of the rabbit and guinea-pig, and of subcutaneous wounds and the blood in order to ascertain the characters of the cellular elements contained therein. The examination was conducted by the cover-glass method first recommended by Koch and Löffler. Eosin, fuchsin, saffranin and methylene blue were used as stains. Some preparations were made by exposing the moist film on the cover-glass to vapours of osmic acid solution.

The examination revealed the presence in the serous moisture and in the tissue plasma of at least two kinds of cells. The one kind resembled in all respects the leucocyte, the colorless cell of the blood. This kind was in fact indistinguishable from the leucocyte.

A second kind of cell was also present and sufficiently numerous. This cell, which is much larger in size than is the former kind, when fixed by osmic acid vapour, often presents a discoid figure, some 30μ to 40μ across; not infrequently however, and more frequently than not when fixed by plunging into osmic acid solution, or by drying, as in the Koch-Löffler method, the cell outline is irregular, often angular, with especial prominence of one angle or of two; in the last case the cell might be described as fusiform. Whatever the shape of the body of the cell may be, there always lies within it, generally towards the centre, a large oval vesicular nucleus, itself somewhat larger than the red corpuscle of the blood. It does not stain so darkly as does the nucleus of a leucocyte. The substance of the flattened plate-like cell-body is markedly granular, particularly so when prepared by the rapidly drying method. The protoplasm reduced to a flattened flake as it generally is, may be of such tenuity near the margin that in the fresh condition and

in many osmic acid preparations the outline of the cell is somewhat difficult to distinguish. The determination of the limits of the cell is however rendered easier by the fact that the cell substance is very granular, the granules taking a sepia tint with osmic acid, and being readily stained by fuchsin and other aniline colours. A marked characteristic of these cells is their tendency to occur in masses and clumps. In the masses the outlines of the individuals are often hard to recognise. In specimens examined fresh upon the warm stage the granules are many of them brilliant and highly refracting, though not to such an extent as are fatty particles. Granules of various size exist in one and the same cell.

FOUR HOURS.—The shortest sojourn we allowed the chambers was four hours in the subcutaneous tissue. The chambers were found to contain fluid with a few blood corpuscles, not collected into rouleaux, and unaltered in appearance. No fibrin had appeared. In short, mixed with the bouillon was simply a trace of blood, as yet unclotted.

Within the chamber in the neighbourhood of its opening were however a large number of leucocytes, unmixed with red blood corpuscles or indeed with any other kind of cell. None of these wandering cells had apparently penetrated far into the recess of the chamber, because there appeared an obvious gap between the position of their pioneers and the diluted blood elements occupying the chamber elsewhere.

NINE AND A HALF HOURS.—Nine and a half hours after insertion into the abdominal cavity, the diluted blood was also found unclotted—although in this instance the chamber had been filled not with bouillon but with water sterilized. The trace of blood which almost unavoidably found its way into the chambers at the time of their insertion had remained unclotted in all specimens examined earlier than fifteen hours. The bouillon containing peptone, seemed, as indeed one might have expected, to retard the clotting of the blood that entered the chamber. In chambers filled with nutrient peptone-bouillon no clot was ever found earlier than twenty hours after the original implantation. On the other hand we found a very considerable formation of fibrin in a chamber that had been filled with sterilized water and then allowed a sojourn of eighteen hours in the subcutaneous tissue. The nutrient bouillon contained commercial peptones, and we think the peptones may not so rapidly diffuse but that this retardation of the clotting may be explained by their presence.

In the early specimens of fibrin production in the tissue plasma

which filled the chambers we had abundant examples of the formation of fibrin filaments as fine straight or very slightly curved lines, irregularly radiating from granular nodal masses. The nodal granular débris, under various reagents, appeared to consist, as the generally accepted view affirms, of altered blood-platelets, and leucocytes undergoing alteration.

About the nodal points of the network of fibrin the leucocytes were grouped. Both on the warm stage and after fixation by osmic acid these leucocytes displayed only rarely any deviation from the spheroid form. There were indeed some instances in which they presented a fusiform outline, or possessed a tiny process jutting from the cell body. Such examples were extremely sparse, and occurred only in the neighbourhood of the opening of the chamber, and in certain situations to be specified immediately. For the most part the cells seemed in an inert condition, as far as one can judge of their activity from their form. In the short time between extraction of the chamber from the body and the fixation in osmic acid or the observation of the cell upon a warm stage under the microscope, their vitality had suffered sufficiently for the cell to have assumed its zero of shape, the subspheroid figure. Or for some reason existence within the Ziegler's chamber was not conducive to activity of the protoplasm, yet from what we shall relate there is no good reason for thinking that the leucocytes within the chamber are in a very different state from those invading the inflamed tissue without. The latter supposition is favoured by the fact that the larger cells, plasma-cells as we shall term them, also found in the chambers under similar circumstances, although subjected to the same *technique* of preparation as these leucocytes, showed well-marked amoeboid movements.

Indeed if the latter of the two suppositions just suggested be not accepted, it becomes necessary to assume that of these two kinds of cell the leucocyte is much the more perishable and delicate, and was practically annihilated by a simple procedure that did not appear to interfere with the vitality of its co-occupant the plasma-cell.

Here must be mentioned another sign of degeneration in the leucocytes examined in these chambers[1]. Many of them showed the triple and multiple nuclear bodies that are universally regarded as evidence of the lethal disintegration of the nucleus—as Fleming names it, the "fragmentation" of the nucleus. On the other hand the cell-

[1] Kuss, Paris, 1846. Pagot, *Surgical Pathol.* p. 151.

body of the leucocyte was not granular or fatty, but fairly evenly though deeply tinted by the osmium. These points are seen in Fig. 1, Plate XXXI.

EIGHTEEN HOURS.—In chambers removed after the appearance of fibrin within them, but before the stay within the body had exceeded eight and forty hours, it was usual to find a number of areas in which leucocytes were present in much greater numbers than elsewhere. Fig. 2, Pl. XXXI.

The tendency to collect to certain points which the leucocytes evinced in even very early specimens was more marked in these later preparations. About the nodal points of the fibrinous network crowds of them were present. The outlying individuals were frequently arranged in lines along the converging filaments of fibrin. The older within certain limits these films of coagulum the more obvious the aggregation of the leucocytes into certain groups. For convenience on account of their prominence and apparent importance in subsequent stages we have been accustomed to refer to these groups shortly as the cell-islets. Cf. Fig. 3, Pl. XXXI. They are little collections of cells, occurring constantly, scattered about in the thin cellular membranes which grow over and within the glass chambers. Some are obvious to the naked eye, especially when the film has been treated with carmine or with haematoxylin, which show them as deeply colored points. They vary in size from a small pin's head downward. The larger islets are often compounded of smaller ones. The smallest display best what we believe to be the structure originally characteristic of all:—a centre of amorphous albuminous débris surrounded by leucocytes; less frequently one or two altered red blood corpuscles form the centre.

It was in specimens of this date that the first evidence of the presence of another cellular element than the leucocytes and red cells of the blood was found. Cells similar to the large flattened plate-like forms of the peritoneal moisture, already adverted to, began to be found in the chamber. The time of their advent varied within narrow limits when the chamber rested in the subcutaneous tissue; when in the peritoneal cavity there was much greater variation in respect to time. This we believe was due to the chamber not coming to rest in one particular spot for some time after introduction into the abdomen. The movements of the viscera seemed able to shift it and prevent its forming adhesions. When put into the abdomen we always placed it about an inch to right or left of the little wound in the linea alba through which it was inserted. But we never found it anywhere

near that situation after a sojourn of more than a few hours. In the course of four and twenty hours the chamber had nearly always passed toward or actually into the position which it almost constantly came permanently to take, that is, a little distance from the median line in front of the psoas muscle at the very root of the mesentery. Once there it appeared in a few hours to contract adhesions, and become fixed in a permanent fashion. Until bound down by adhesions, the full complement of cells did not reach the interior of the chamber.

In one instance, in a chamber exposed for eighteen hours in the subcutaneous tissue, plasma-cells were found in considerable numbers near the opening. They were indistinguishable in appearance from the plasma-cells of the normal subcutaneous tissue, except that a greater variety of individual form was to be seen in them. In later specimens the plasma-cells were found scattered throughout the whole chamber, although most numerous near the opening.

SEVENTY-TWO HOURS.—In a preparation from a chamber which had been seventy-two hours in the subcutaneous tissue, plasma-cells entered into the formation of the islets even in the portions furthest removed from the opening. At the opening however no other kind of cell was mixed with them, which was not the case elsewhere. No forms intermediate between the leucocyte and the plasma-cell were to be found; they were repeatedly expected and repeatedly looked for, but the search was unsuccessful.

The preparations gave an almost bewildering number of examples of the infinite variation in shape of the large amoeboid plasma-cells, which also varied very considerably in size, and as to granules. The body of the cell was for the most part plate-like, being in many instances extended into so thin a film that its exact limit was hard to determine, especially when, as occasionally happened, the granules of the cell-body were less pronounced towards the periphery. Some idea of the wide diversity of outline exhibited by individual cells may be gathered from our figures. Cf. Figs. 1, 4, 5, 6, 7 and 8, Plates XXXI. and XXXII.

It must not be thought, however, that in any of its forms the plasma-cell could not be distinguished with certainty from the leucocyte. In the same way as in the peritoneal moisture and in the plasma of subcutaneous tissue, the former is here also on the warm stage and in osmic preparations, characterised by larger size, coarser granules, the constant presence of a single clear nucleus of oval figure, and by the differences in staining qualities and mobility already

referred to. We may here add that these differential characters may be obscured by faulty methods of examination.

In the specimens obtained from chambers that had rested for seventy-two hours in the subcutaneous tissue of the guinea-pig, we found individuals among the plasma-cells, which showed well-marked vacuolation, Figs. 1, 4, 5, Pl. XXXI. For the most part the matter within the vacuole was a granular débris that furnished no sufficient clue as to its nature. But in a few it was indisputable that the vacuole contained, more or less altered but still perfectly easily recognisable, a leucocyte or red blood corpuscle. In Fig. 4 is shown the appearance presented by one of these cells. A large vacuole contains a somewhat faintly stained body, which is finely granular and indistinctly nucleated. It is a little smaller than is the nucleus of the plasma-cell itself. Fine threads seemed to pass from the sides of the vacuole across the cavity to the substance of the included leucocyte. Taken with the context afforded by examination of other cells in the neighbourhood we believe that this and other similar instances were examples of leucocytes lying in vacuoles in the plasma-cells. Many stages of ingestion could be found. Cf. Figs. 1, 4, 5, Pl. XXXI. Simple approximation, the hollowing out of a little bay in the side of the plasma-cell into which the leucocyte was as it were drawn, partial inclusion, total inclusion—all these were exemplified. And further there were many vacuoles in which mere granular débris lay. This débris was, we think, probably the still undigested remnant of the ingested leucocyte or red blood cell. We doubt whether without very special apparatus the cells of the tissues of mammalia can be kept in sufficiently normal condition for sufficient length of time to compass observations on ingestion by living cells; we were however much assisted in the interpretation of the appearances of the osmic fixed preparations by the processes described by Miss M. Greenwood for the Rhizopoda. Her observations[1] were conducted on living specimens of Amoeba proteus and Actinosphaerium, and she was able to follow in these animals under the microscope all the visible phenomena accompanying the ingestion of prey. In our preparations we had as it were a number of amoebae, many of which had been actively engaged in ingesting living prey, immediately before the reagent had been used that killed them so rapidly as to allow no time for any great departure from their previous aspect.

[1] This *Journal*, Vol. VII. p. 253, Vol. VIII. p. 263.

Nor were leucocytes the only bodies to be found within the substance of the plasma-cell. Red corpuscles of blood were recognisable in them. Very frequently along the border of the space in the chamber, the plasma-cells lying in great numbers near the cement (shellac glue) which fixed the strip of tin-foil to the glass, were filled with tiny droplets of oil that became deep black under the treatment with osmic acid. Sometimes the entire cell was dotted, except just round the nucleus, with fine fatty particles of a fairly equal size: sometimes the oil was collected into a few much larger globules. The cement itself turned deep black under osmic acid treatment. There was little room for doubt that the black particles in the plasma-cells were derived from the cement near the cells; whether the cells took up the particles without altering them, or whether the particles were in any degree a food for the cells are points we can give no answer to.

Contiguous plasma-cells or even those a little distance apart were often connected together by their processes (Figs. 1, 5, 7 and 8, Plates XXXI. and XXXII.). The bands of connection might be short thick arms or long gossamer threads of protoplasm. By similar arms and threads the cells seemed to adhere to the most diverse objects in their surrounding. The surface of the cover-glass, a filament of fibrin, a hair, a fibre of cotton, a lump of the cement fastening the sides of the chamber together, all afforded points to which the processes from the plasma-cells would cling (Figs. 14 and 15).

There were present also in chambers of eighteen hours', twenty-two hours', twenty-six hours', forty-eight hours', and seventy-two hours' standing, as also in others of older date containing well formed granulation tissue, many giant cells (Fig. 6)—huge multi-nucleate cells, that obviously in many instances were cell-fusions. Congregations of large plasma-cells as before mentioned were frequently met with. They adhered one to another in groups. And here many collections of them existed intermediate in character between those groups in which the individual cells were agminated but easily distinguishable from one another, and giant cell masses in which the nuclei were the only guides to the individual position of the coherent members. Some appeared to be cell-fusions; many did not. In these latter the nuclei were gathered together into an irregular heap. The ring-like arrangement of the nuclei frequently found in the giant cells of tubercle was never observed in these membranes by us.

Of nuclei in these giant cells there existed apparently two kinds. One was large, clear, and oval, having all the characters of the nucleus

of the separate plasma-cell; it was invariably present in all the giant cells. The other sort was smaller, round, more darkly tinted by osmium treatment, and was not invariably present, that is, did not exist in every giant cell, but was in some cells even more numerous than the larger oval variety. We doubt very much the accuracy of describing the latter smaller bodies as true nuclei. We incline to believe, from their great similarity to some of the leucocytes observed in the plasma-cells, that they are nothing but leucocytes surrounded by the substance of the giant cell and somewhat altered in appearance. Against this supposition is the fact that there was often no indication of a vacuole-space around the ingested cell, but in support of it the substance of the plasma-cell was seen sometimes very closely applied to the ingested leucocyte in instances in which there was very little doubt as to the nature of the included body. In osmic preparations there is generally a light space free from granules immediately around the oval nucleus of the plasma-cell that simulates somewhat closely the appearance of a vacuole about the nucleus itself.

Advancing further into the chamber, in the specimens of more than forty-eight hours' duration, the plasma-cells begin to apply themselves to the islet-groups of leucocytes. Cf. Figs. 8 and 10. They surround the leucocytes. The islets come to consist of a central portion made up of leucocytes, and an outer zone of large and granular plasma-cells. In this way the islets seem to increase rapidly in size. Neighbouring islets appear to become merged together. Giant cells are frequent in them, especially, it would appear, near, although not actually at, the centre. Most of the growth that went on in the membrane appeared to consist in enlargement of individual islets, and the fusion of neighbouring islets. The islets appeared to be the chief growing points of the tissue. But it is true that gradually a more or less continuous sheet of plasma-cells is formed over the intervening space between the islets. When very thin the inflammatory membrane consisted of a layer of scattered cells lying separated by considerable but fairly regular distances one from another. Each individual cell was of a discoid or fusiform figure, and granular, with a large clear nucleus. The edge of the disc was thin and often deeply scalloped; it merged, under all methods of staining used by us, at certain points quite imperceptibly, in a tenuous film which composed the bulk of the membrane proper. When fixed with osmic acid and after-stained with haematoxylin (Ehrlich's), this membrane is shown to contain, if not to be entirely made up of, a feltwork of filaments, like filaments of fibrin. These

cross in every direction in the plane of the membrane, without prominent arrangement in any one particular sense. The individual filaments vary a good deal in size. Fig. 16, Pl. XXXIII.

It was among the plasma-cells of the fringe of the islets that we noticed the earliest regularly fusiform cells, the immediate precursors of fibrous elements in the new tissue. It is true that plasma-cells of an irregular spindle-shape were observable not rarely among even the earliest of the plasma-cell swarm entering the chamber. But in those instances the outline was probably but one of many which the amoeboid cell successively assumed, and generally it was not of the same character as the regularly fusiform type prevailing among these plasma-cells in the outskirts of an islet. In that latter the majority of the cells lay in lines concentrically set about a core of ill-stained, broken-down matter that composed the centre of the mass. Cf. Fig. 11, Pl. XXXII. The fusiform fibroblasts began in fact the encapsulation of the *débris* of the breaking-down blood cells, &c. The lengthening out and assuming of a regular spindle form took place also very early in those cells that had become attached to hairs and cotton-fibres, and lumps of the shellac glue. They were soon found adhering there in rows of regular disposition, the rows consisting entirely of typical young fusiform fibroblasts.

Later than seventy-two hours.

Older specimens revealed further progress in the formation of a fibrous-tissue membrane. After a stay of eight days, or ten days, or fourteen days in the subcutaneous tissue in many instances the islets consisted of plasma-cells alone. The leucocytes had disappeared. The pigmented remnants of the red blood corpuscles were much longer traceable. In many places along certain lines the spindle-shaped cells had become attenuated, and formed distinct bands and often long and delicate cords (Figs. 12, 13). In many places in the tenth day specimens, and in some of the eighth day ones an inter-cellular substance showing fibrillation exists (Fig. 12). This extra-cellular matter is well seen where, as occasionally happens, a single chain of fusiform fibroblasts, set in end-wise series, has produced a thread-like tiny cord. Each fibroblast appears to lie in a sheath of fibrillated matter. The delicate lines marking the fibrillae run parallel to the contour of the cell. The fibrillated matter was not tinted by osmic acid or by any of the stains employed by us to the same depth as the granular substance

of the cell itself. The granules of the cell-body, the clear oval nucleus, were still marked characters of the plasma-cell, although it might be considered at this stage to have become a fixed corpuscle of connective tissue.

We were unable to satisfy ourselves on the question as to whether the fibrillated extra-cellular matter had been formed by direct transformation from the surface portion of the cell-body, or whether it had arisen as a secretion from the protoplasm of the cell. But the latter view appears to us the most probable, if only for the reason that the fibroblast-cell and its new capsule of fibrillated matter are when taken together much larger than, so far as we have observed, the individual naked fibroblast ever is.

From the islets the bands of spindle cells spread away in various directions. The determination of the direction of the earliest-formed chains of spindle cells seemed to us greatly due to the lines taken by the filaments of the original fibrin-network; the radiation from the same nodal points, the interlacing not always at acute angles but frequently in rectangular fashion.

In membranes of ten, fourteen, and even eighteen days' growth, not all the cells nor even the majority were spindle-shaped. A vast number were triradiate, and multiradiate; some had but one process; very few were rounded. Many recalled to mind the branched fixed corpuscles of the cornea. Long tapering branches united cell to cell, not only the cells of one plane one with another, but the cells of different planes also (Figs. 8, 9 and 17). A meshwork of infinite variety and complexity was thus established. But in all these examples of plasma cells in the stable as well as in the previously described labile forms, the granular nature of the cell substance and the clear oval nucleus were characters never lost.

In the same manner as did the more delicate strands of fibrous tissue, larger, broader sheets and beams arose. In all the spindle cells side to side as well as end to end are separated by intervening matter fibrillated in a direction parallel to the longer axes of the cells.

It may have been noticed that no mention has been made of any developing blood vessels in the membranes examined. It is a striking fact that in none of the preparations, not even in the preparations of eighteen days' growth, taken from the peritoneal cavity, did we find in any instance any trace of a formation of blood vessels. Nowhere were capillaries to be found; although the chambers were bound by adhesions and in the later specimens encapsuled in cicatricial tissue. This obser-

vation seems to furnish a negative to the view advanced by Creighton that the giant cells of granulation-tissue are exclusively vaso-factive. Here we had giant cells in abundance, but never any capillary formation. Perhaps the film of tissue in the chamber was thin enough to allow sufficient nutriment to reach the cells by fluid soakage only.

Abstract of some of the Notes of the Experiments.

The number of hours mentioned corresponds to the time during which the chambers rested in the bodies of the animals.

1. 4 *hours.* Subcutaneous. No fibrin. No roulcaux of red cells. A large number of leucocytes in the neighbourhood of the mouth of the chamber.

2. 18 *hours.* Subcutaneous.

High power. Fibrin network very extensive. Crowds of leucocytes at the nodal points of fibrin. Cells circular in outline, nuclei crescentic or trilobed. At the periphery of the islet a few leucocytes still in an active state and of irregular form. At the mouth of the chamber are a few large plasma and giant cells. Fig. 2, Pl. XXXI.

Low power. The islets of cells in the fibrin film are well seen. Indeed they are visible to the naked eye, about the size of pins' heads.

3. 72 *hours.* Subcutaneous.

Islets more marked. Fibrin network very extensive. Numerous large plasma-cells encircling the islets of leucocytes at the nodal points. In the neighbourhood of the mouth of the chamber the plasma corpuscles are more numerous, and the islets are partly made up of these cells. Moreover, numerous red and white blood cells are visible in the vacuoles of giant cells and in those of separate plasma-cells.

72 *hours.* Peritoneal cavity.

The chamber was quite *free.* No trace of an adhesion.

The chamber seems to have escaped the leucocytic immigration. The islets are formed almost entirely of plasma-cells.

4. 8 *days.* Peritoneal cavity. Fixed by an adhesion; how long fixed?

The islets are formed of plasma-cells alone. The leucocytes have all disappeared, a few only are visible in the vacuoles of the larger cells. The cells at the circumference of the islets are lengthened out along the lines of fibrin, and joined with others free of the islets to form a giant cell field, or plasmodium. Every cell is connected

by long processes with others, so that looked at in one way the whole field is one giant cell. The islets consist often of one giant cell together with numerous plasma corpuscles. It is only at the periphery of the islets that the contour of the individual plasma-cells can be made out. See Fig. 8. Foreign bodies such as blood-clot, shellac, etc. are surrounded by a capsule of spindle cells. Fig. 11.

5. *Several chambers.* 8 to 18 days in peritoneal cavity. Some fixed, and some *not* fixed by adhesions.

Fibrillation advanced. Every stage can be observed, from the simple fusiform elongation of the plasma-cell to the development of a perfect fibril. Fig. 12.

The above experiments seemed to point to a certain definite period at which the migration of leucocytes and connective tissue corpuscles occurred.

In order to examine somewhat further the behaviour of the leucocytes and of the plasma-cells respectively toward the chamber, a slight modification of the mode of experiment was used on two occasions. Two chambers (or four) were placed side by side in the subcutaneous tissue. At the end of twenty-two hours they were taken out, one was dropped into osmic acid, and the other was sealed with warm paraffin. The sealing was done by dipping the mouth of the chamber into a soft paraffin melting at 107° Fahrenheit. Only the part of the chamber immediately next the opening was touched by the paraffin, which was just above the temperature of solidification. After being sealed the chamber was placed in the abdominal cavity of a second guinea-pig, there to remain for incubation.

In this way it was possible to compare the contents of two chambers which had been placed side by side in the subcutaneous tissue, and whose contents were presumably the same at the time of withdrawal. One was then fixed for histological examination. The other incubated for a longer period, no new cells being allowed to enter during this second incubation.

The imperviousness of the chambers after sealing was tested in the following two ways:

(*a*) A little 1% hydrochloric acid was introduced in the chamber, the outer edge of the opening carefully dried, and then the paraffin applied by dipping the mouth just as in the experiment above. The chamber was then placed in blue litmus. No change took place in the litmus, although the chamber remained a week in the solution.

(β) A little of an active culture of Spirillum Finkleri was introduced into the chamber, the edge of the opening cleaned, and then sealing performed as before. The chamber was then placed in nutrient broth (in another case in nutrient gelatine) for a week, at a temperature of 35° C. No growth appeared in the broth (or gelatine). In a control tube the broth was turbid in two days.

In the experiments performed in this way the appearances observed in the chambers were alike. The chambers were withdrawn at the end of twenty-two hours, one was then incubated further for another forty-four hours.

The contents at end of twenty-two hours were as follows, viz.:—
a large number of leucocytes and several patches of red corpuscles. Plasma-cells are also present but very sparsely; they are most numerous at the mouth of the chamber. They are scattered at long intervals. In one place a few plasma-cells are collected around some red cells, and a fibre of wool. Fibrin filaments are present in the chambers taken from the rabbit, but none in those from the guinea-pig.

Contents of sealed chambers after forty-four hours' further incubation:

Fibrin network extensive. The leucocytes lie around the red cell masses. The leucocytes possess for the most part "fragmented" nuclei. The plasma-cells are far more numerous. They exist in patches and groups quite apart in many cases from the clots or the leucocytes. Many plasma-cells lie mingled with the leucocytes around the little blood-clots.

Remarks.

We have pointed out that there appears to be a definite sequence of events in the processes induced within the tissue by implantation there of the experimental chamber—processes which must according to ordinary terminology be designated as inflammatory in nature. At a definite time and in a definite order occur the immigrations respectively of leucocytes and of the daughter cells of the tissue corpuscles. The former had commenced at the end of four hours. In our experiments the fibrin within the chamber was crowded with leucocytes within eighteen hours of the time of insertion in the subcutaneous tissue of rabbit or guinea-pig. But in those eighteen hours scarcely a plasma-cell could be found to have penetrated into the chamber. On the other hand after the lapse of seventy-two hours the nodal points, which had been previously the centres of aggregation of leucocytes, had been

transformed into islets consisting chiefly of plasma-cells. This primary leucocytic invasion and the subsequent appearance of "larger cells with clear vesicular nuclei" has also been noted by Ziegler and by others. W. Hunter[1] passed by transfusion all the blood of one rabbit into the peritoneal cavity of a second. At the end of a few hours he was able to find scarcely a white corpuscle in the circulating blood; the amoeboid cells had migrated into the peritoneal cavity where the foreign body—the fluid blood or the coagulum—was resting.

This observed order in the occurrence of events serves to explain "the periods of repose" that are known to the surgeon. The fluid which oozes from the surface of a wound is at first blood tinged, but soon becomes pale, until at the end of a few hours the surface is covered with a whitish film. This film is a fibrinous network, containing within its meshes leucocytes in enormous numbers, and ever increasing as the first few hours pass by subsequent to the development of the film. "Such a calm continues from one day to eight, ten, or more, according to the nature and extent of the wounded part, and the general condition of the body." "The calm may be the brooding time for either good or evil; whilst it lasts the mode of union of the wound will in many cases be determined." "Moreover in open wounds the time at which on each tissue granulations are produced is determined by this calm; for they begin to be distinctly formed at its end[2]." The share which we think the white corpuscles have in the constructive process of repair will be evident from what we mention elsewhere in the paper. "Apparently they do not hinder it[3]." And previous to the advent of aseptic surgery it was believed by many that to leave the cut-surfaces of a wound exposed until they bore a whitish, glassy film, and not to put them into contact until then, was to give a condition favorable to union by primary adhesion.

Indeed, whatever view be adopted regarding the fibroblastic value of the leucocyte, certain other purposes which it may subserve in the process of repair were in our experiments extremely obvious. It was the pioneer of all the wandering swarm of cells that visited the intruding occupant of the tissue. Whatever causes, intrinsic or extraneous, guided its early voyaging, the route it traversed and the position it

[1] *Journ. Anat. and Phys.*, Vol. XXI., 1887. "In 6 hours scarcely a white corpuscle was to be found in a field of several hundred squares (instead of 4, 5, or 6 in every 100 squares) though the red cells were much increased in number."

[2] Paget, *Surgical Pathology*, Ed. IV. p. 151.

[3] *Ibid.* Paget.

assumed seemed to determine almost absolutely the course of after-coming plasma-cells that appeared in great measure to be simply followers along the track thus broken for them. Where the intruding body was of penetrable nature, as in the case of blood-coagulum, these leucocytes entered it in the van of a destroying army, that in turn attacked it from channels that leucocytes had prepared. By leucocytes the mass to be absorbed was in part previously divided up and made to offer a greater surface for absorption by plasma-cells. Where larger masses of clot are concerned cracks and fissures occur from chemical causes, as shown by one of us elsewhere (B.)[1], which in the same way allow of the entrance of the plasma-cells among whose functions in the clot mass are absorption and substitution. The filaments of fibrin when they were present appeared to direct to a certain extent the path travelled by the cells. Certainly to group themselves about the granular nodal points of the fibrinous network was quite characteristic of the distribution of the leucocytic swarm, and this directly influenced the formation of islets in the cellular membrane, the islanding being the direct outcome of the original grouping.

And leucocytes served also as a pabulum for the active plasma-cells[2]. Just as, in the extremely interesting observations given by M. Greenwood[3], little monads, Euglenae and Algae coexisting in the same water with Amoeba proteus were by it ingested, so leucocytes become the prey of the plasma-cell, and are by it included and ingested. And if the growth and proliferation of the plasma-cells be of importance in the

[1] Erasmus Wilson Lectures of the present year.

[2] The plasma-cells are considered by Metschnikoff to be among his group of "phagocytes." He writes: "Die weissen Blutkörperchen bilden einen allerdings ansehnlichen Theil aus der Summe der Phagocyten, indessen gehören zu diesen, wie ich in meinen sämmtlichen Arbeiten ausdrücklich erwähnt habe, auch amöboide Bindegewebs-zellen und manche andere zellige Elemente." *Virchow's Archiv*, Vol. CVII. p. 239, 1887. He proposes to call "grosse in der Regel mit einem einfachen (nicht gelappten) Kerne versehene Phagocyten," in which "der Kern ist rund oder häufiger oval," by the name Makrophagen, no matter what their origin may be. This in contradistinction to the Mikrophagen, "mit stark tingirbaren, zum grossen Theil gelappten oder fragmentirten Kernen und sehr blassem Protoplasma," which are for the most part, he affirms, leucocytes. The plasma-cells are therefore included in his Makrophagen. Cf. also Wyssokowitsch, *Koch's Zeitschrift*, Bd. I. Lief. 1, p. 39, 1886. Metschnikoff studied the phagocytic power of plasma-cells in subcutaneous tissue in cases of Erysipelas in the human subject. In some preparations we have obtained lately in the Ziegler chambers from an experiment in which the wound was allowed to suppurate, there are abundant instances of bacteria within the plasma-cells. Cf. also Hess, *Virch. Arch.* Bd. CXIX. Hft. 3, on "Gland cells destroying bacilli."

[3] This *Journal*, Vol. VII. p. 253. Vol. VIII. p. 263.

process of repair, what circumstance more propitious than the presence in abundance of nutriment so delicately adapted and so highly organized as the substance of the leucocytic cell? Of Amoeba and Actinosphaerium it was remarked that the food most suitable to these forms is unshielded non-coagulated proteid matter. A low degree of vitality, a diminished activity of its protoplasm, renders an organism easier prey, more readily captured and more readily absorbed. The plasma-cell may in some respects be taken as a hothouse variety of amoeba; it finds its unshielded non-coagulated proteid in the dead or dying leucocyte. It will be remembered that within the chambers the leucocytes revealed striking signs of lowered vitality.

Not that the number of instances in which we could detect an actually included or a partially ingested leucocyte would, we think, account for the large disappearance of them that does actually occur. Is it not probable that the plasma-cell can exert digestive action upon material which it does not incept? Suppose a proteolytic ferment secreted by the plasma-cell, and leucocytes that are dead or dying as in the above experiments; a gradual solution of their substance in the tissue plasma will occur, yielding to it an abundance of rich food for other cells that are in a thriving condition.

Passing in review the chief points observed in regard to plasma-cells, it became clear enough to us that in the study of their origin and development lies the best key to the problems of the formation of tissue of repair. We found them traceable up from forms of an amoeboid kind, different in many ways from the amoeboid cell-forms of blood and lymph, through individual types of almost endless diversity of figure with the utmost variety of combination and interdependence, onward finally to the fixed corpuscle of fusiform or of stellate shape imbedded in fibrillated material.

As to giant cells, often it was obvious that the large cell had resulted from a fusion more or less complete of the bodies of several smaller cells, the nuclei of which remained distributed regularly through the substance of the aggregate. In other instances a massing of the nuclei of the giant cell about one point appeared to denote a mode of origin from a single cell that had grown and undergone nuclear multiplication without actual separance of the daughter cells from the parent as they had been produced.

Again, by the union of cell with cell, by means of long pseudopodium-like processes, it was sometimes found that a whole field under the lens was occupied by the net-like ramifications of one huge multi-nucleated

cell—better described perhaps as an unbroken sheet of anastomosing cells. The characters of the giant cells in the implanted Ziegler-chambers resembled in this particular those of such giant cells as occur in marrow, growing bone, the splenic pulp, myeloid sarcoma, and in granulation tissue. In no cases did the arrangement of the nuclei in them bear resemblance to the ring-like or other regular disposition often seen in the giant cells of tubercle.

Upon the position of the giant cells depends partially the arrangement of the fibrillated tissue which is ultimately produced. The run of the bundles of fibrillae is often from and between giant cells. The cells range themselves previous to fibrillation in lines spreading for some distance from the giant cells; in fact in many ways the resemblance of giant cells to cell-islets is a close one. Just as in some cases, if not in all, the so-called giant cell is really but a congeries of smaller coherent cells, attracted to one and the same spot for the purpose of participation in a common prey, so is it with the cell-islets also. The groups of leucocytes from which the cell-islets arise appear to be originally formed under the common attraction which is offered to these cells by the albuminous *débris* present at the central nodes of the fibrinous network. Later, the leucocytes themselves becoming from some cause or another effete and of low vitality, exert a similar attraction upon the wandering plasma-cells, and afford to them a rich and easy quarry. By this arrival of fresh cells the islet is increased in bulk. The more centrally situated individuals feed upon the leucocytes they have surrounded, and the latter rapidly merge to an amorphous kernel for the entire mass.

The outlying cells become disposed along definite lines, and as it were sketch in in its main outlines the general plan which the adult arrangement of the new fibrous tissue will display.

The cell-islets are the centres of most active growth and proliferation in the young cellular tissue. They contain the stores of nutriment that are gradually dissolved and digested. They may contain also innutrient matters, and matters such as are not only innutrient but incapable of solution by the cells or plasma. At first the shape of those cells which are immediately next to the kernel of nutritious matter in the islet is irregular, and suggests amoeboid properties in the cell; later the cell becomes almost regularly fusiform, and is applied by its side to the material which gradually disappears. The material comes to be encircled by chains of fusiform cells set concentrically around it. It becomes encapsuled in the same way as is the ligature placed around an

artery by the surgeon, or as is any foreign body placed within a wound which heals around it. The fusiform fibroblasts slowly exert the same solvent action upon the imprisoned material as did their amoeboid ancestors. No doubt the more easily affected portions of the material are the first to go into solution and disappear, leaving a constantly less amenable residue and a less nutrient one; and perhaps it is in accordance with the decreasing supply of food from this source that the cells in contact with it undergo gradual change and lose their pristine elasticity of form. They assume the spindle-shape, and a fibrillated intercellular cement substance comes into existence between them. We have already seen reason to think that this "matrix" is a secretion from the cell. Prominent among the conditions under which the young fibroblasts begin to form it is, it would seem, a diminution in the amount of pabulum at hand to support growth. Much as amoeba under adverse conditions assumes an encysted form, so where food is scanty do the inherited tendencies of the fibroblast lead it into states of quietude and encystment. The less nutritious, the more inert the foreign body which the plasma-cells surround, the sooner do they become fixed cells, the earlier do they elongate, and make around themselves the bed of fibrillated matter, which commits them to immutability of form. In the same specimen in which plasma-cells preying upon remnants of blood-clot were still actively amoeboid, it often happened that around innutritious matter as hairs, and cotton fibres, the cells were already perfectly developed into young fibrous tissue.

When embedded in the fibrillated secreted substance all digestive and absorptive activities within the cell do not cease. Encapsulation does not arrest absorption. This has been shown by one of us (B.)[1]. It holds even in those instances in which the foreign substance is of such a nature as to resist digestion or chemical solution for a lengthened period. Carbolized cat-gut is quickly split up and destroyed by an environment of living tissue; kangaroo tendon, which is denser and more resistent, becomes encapsuled and continues to be gradually dissolved and absorbed after a dense fibrous investment has been produced around it; it may require a hundred days for complete disappearance. Prepared silk in like manner becomes encapsuled, but is finally absorbed in a period which sometimes is as lengthy as three years. Even silver is slowly destroyed. Gold and platinum appear able to resist indefinitely long.

[1] Erasmus Wilson Lectures of the present year.

Unfortunately it was only until the present experiments had been concluded and the present paper very nearly so that we were able to obtain copies of Professor Ziegler's monographs from the Würzburg Institute. We had been obliged to satisfy ourselves with the results of his work in abstracts of the original papers. As a matter of fact our work has not been a repetition of his quite to the extent we had imagined. A great part of our observations deal with periods which his do not touch or only slightly so. [1]His first communication is based upon observations on chambers implanted in sixteen dogs at thirty-six different intervals. But of these only five, upon four individual animals, refer to the first two weeks after implantation, and he records no observations prior to the seventh day. Of our observations with rabbits the major part refer to the first two weeks after implantation, and our earliest observations were made only four hours after implantation. We imagine too, judging from the beautiful illustrations to the original papers, that the cell-masses that we have so frequently referred to as cell-islets are included by him among the giant cells. It must be remembered also, that his experiments date prior to the acceptance of antiseptic surgery, and eleven times he records pus, either in the implanted chambers or in the wound. In no case did we ever find the slightest trace of pus, as we have said already.

It will have been seen that in most points our observations entirely confirm the original observations made by Ziegler. One particular there is however, and that one of fundamental importance, in which we are in disaccord with the descriptions furnished in his paper. As far as we observed, there are in the tissue-plasma of a part subjected to irritation such as that described in the experiments two kinds of cell. On the one hand there are present leucocytes indistinguishable from and probably identical with the colorless corpuscles of the blood; on the other hand are plasma corpuscles, cell-elements proper to the connective tissue of the part offended. The cell that plays as we incline to believe the only actively constructive *rôle* in all the energetic upbuilding of new tissue that goes forward in the part, is the plasma-cell, a corpuscle absolutely distinct from the colorless corpuscle of the blood. Our cover-glass preparations lead us to believe that these free cells in small number exist in the tissue plasma even under normal circumstances. Where the connective tissue corpuscles are proliferating, as for instance within an inflamed area, there these free

[1] Ziegler's second communication deals entirely with "die Schicksale der eingewanderten, zum Theil bereits veränderten Zellen von dem 25. bis 70. Tage."

tissue-cells are enormously more numerous. In our experiments, out of them arose the permanent membranes, to be designated inflammatory if the ordinary unsatisfactory use of the term be sufficient, which spread themselves over and inside Ziegler's chambers when lying in a subcutaneous space or in the peritoneal sac—membranes composed at first of cells entirely similar to the corpuscles of the normal tissue-plasma. Colorless blood-cells doubtless wandered in the surrounding of the chamber, and doubtless entered in plenty the space within it. But these leucocytes had no permanence of possession. The fibroblast of the new tissue was not of leucocytic origin. Our observations yield no support to Cohnheim's view of the genesis of cicatricial tissue from leucocytes.

When pus is formed in an inflamed focus many of the migrated colorless corpuscles of the blood become, as is well known, pus-corpuscles. It does not appear strange that where pus is not produced those of the swarm of leucocytes, which do not drain off by lymphatic channels from the tissue they have temporarily invaded but remain behind, should not thrive within it. Many circumstances might, we conceive, render their sojourn perilous. The high carbonic acid tension, the comparatively stagnant character of the fluid, the presence of, to them, unwonted chemical bodies, and of others in unwonted percentages —these are instances of conditions which might, we conceive, constitute an environment of disadvantage. And that the migrated leucocytes should rapidly be not merely acclimatized in the new locality, but should actually become fixed elements of the part, and generate the cells of a fibrous tissue is to our minds improbable. The cells of fibrous tissue and the colorless corpuscles of the blood are both of mesoblastic origin, but we have no evidence that they are more nearly related one to another than are the fibres of a striated muscle to the endothelial cells of an artery. No one advances the view that of these latter one can by any means be made to reproduce the other. Even in tumours with their apparent departure from the normal type of growth the principle of heredity is in reality religiously obeyed. "The secondary growths in carcinoma are identical with the primary, for it is the epithelial element which is infected, and it is this element which determines in the normal process of development the general anatomy of the parts around, be they glandular or otherwise. So a columnar celled carcinoma of the rectum produces in its metastatic growths intestinal crypts in the liver; a thyroid cancer produces in its secondary tumours thyroid tissue in the bones; an osteoid sarcoma shows in its

secondary manifestations osteoid tissue; and it might even be conjectured that if the epithelium over the papilla of a hair received the carcinomatous infection hair-like structures would be found in the primary and secondary tumours[1]." In the production of scar-tissue it seems to us of transcendent significance that such tissue is characterised by the possession of cells of which each tends to secrete a collaginous capsule for itself, so that around the cells a more or less solid and fibrillated intercellular matrix comes to be characteristic. Cells with a similar tendency characterise broadly the connective tissues wherever found. It is in accordance to laws of natural descent for the cells of connective tissue, when thrown into renewed and extraordinary genetic activity in what is termed plastic inflammation, to produce a progeny of cells possessed of the same tendencies as themselves. And among all these tendencies which one is more unfailingly repeated in them than to mould a semi-solid fibrillated collaginous capsule, in short, to build up fibrous tissue? But the cells of the blood nowhere show signs of any such propensity. The colorless corpuscle of the blood is conspicuously endowed with a character apparently opposed to, even incompatible with, the formation of a semi-solid circum-cellular test—the whole story of its normal life so far as we know that, is associated to one continuous flux of form.

The term "inflammation" is at present employed to signify a number of phenomena of which some are not only widely dissimilar one from another, but are even not necessarily associated. John Hunter recognized the importance of distinguishing two classes of injuries to tissue, in consequence of the radical differences between the resulting processes of metabolism set up by them in the tissue and comprehended within the one term inflammation. He says[2], "The injuries done to sound parts I shall divide into two sorts, according to the effect of the accident. The injuries of the first division in which the parts do not communicate externally seldom inflame; while those of the second which have an external communication commonly both inflame and suppurate." Modern surgery[3] has shown how right Hunter was. Would it not be well to designate by separate titles the parts of the inflammatory process due to the plasma-cell of the tissue, and to the migrated colorless corpuscle of the blood respectively? By

[1] See a discussion of this question, *Path. Soc. Trans.* Vol. XXXVIII. pp. 423 and 424, 1887. Ballance and Shattock, "Cultivation experiments with Cancer."

[2] Quoted from Paget's *Surgical Pathology*, Ed. 4, p. 131.

[3] *Ibid.* p. 130.

such a terminology the destructive and suppurative processes of inflammation would be sundered from the constructive and reproductive. This would obviously be of advantage, if, as would appear likely, pathology is to teach that pus is an adjunct of the inflammatory process only when the irritation produced within the tissue is aggravated by the associated presence of a sufficient dose of bacterial virus.

REFERENCES TO PLATES.

Plate XXXI.

Fig. 1. Contents of experimental chamber that had remained 72 hours in the peritoneal cavity of the rabbit. Five large amoeboid plasma-cells, with altered red corpuscles and apparently dead leucocytes. Outlined with camera lucida. Apochromatic oil immersion and ocular No. 4. Zeiss. Prepared over osmic vapour.

Fig. 2. Contents of a chamber for 18 hours in the peritoneal cavity (rabbit); near the centre of the chamber. Fibrin filaments, leucocytes, red corpuscles, and an ill-defined granular mass forming a nodal point in the fibrinous network—the beginning of a "cell-islet." Outlined under camera. Similar method of preparation, and similar magnification to preceding.

Fig. 3. Fragment of inflammatory membrane formed within a chamber placed for three days in the subcutaneous tissue (guinea-pig). Islets and groups of islets scattered through the membrane. Zeiss, Obj. A, Oc. 2. Osmic acid solution, and Ehrlich's logwood.

Fig. 4. Contents of same chamber as in Fig 1. Close to the opening of the chamber. Five plasma-cells, one of them continuing a leucocyte within a large vacuole. Magnification and method of preparation as in Fig. 1.

Fig. 5. Contents of same chamber. Two plasma-cells and two red corpuscles; the plasma-cells are indistinguishably united with fine filaments of fibrin in their surrounding, some of which are given in the figure. Osmic acid vapour. Zeiss, apochr. system, oc. No. 2.

Plate XXXII.

Fig. 6. Giant cells from chamber 72 hours in the peritoneal cavity (rabbit). Zeiss apochr. system, ocul. 5. Osmic acid vapour.

Fig. 7. Plasma-cells from same preparation which furnished Fig. 6.

Fig. 8. "Cell islet" from inflammatory film obtained in a chamber left eight days in the subcutaneous tissue of the guinea-pig, At the margin it is united to outlying plasma-cells. Zeiss oil, oc. 4. Osmic acid vapour.

Fig. 9. Young cicatricial tissue of anastomosing branched cells, some of which are represented under the higher magnification in Fig. 17. From a thrombosed artery (syphilis) near the centre of the thrombus. Zeiss A, oc. 3. Logwood. Preparation kindly shown us by Dr Seymour Sharkey.

Fig. 10. "Cell islet" from inflammatory membrane obtained from chamber five days in the peritoneal cavity of the rabbit. Osmic acid solution. Zeiss oil imm. and oc. 2.

Fig. 11. Mass of blood-cells (? clot) surrounded by fibroblastic cells, and invaded by them at four places. Inflammatory membrane from chamber eight days in subcutaneous tissue. Magnification as in preceding, and prepared in similar manner.

Fig. 12. Fusiform plasma-cell (fibroblast) surrounded by a fibrillated material which forms a thread-like band of connective tissue. Zeiss oil and oc. 4. Osmic vapour. From chamber 10 days in subcutaneous tissue.

Fig. 13. Similar but larger and thicker fibrous band from same preparation. Similar preparation and magnification.

Plate XXXIII.

Fig. 14. From chamber five days in subcutaneous tissue. Plasma-cells adhering to a cotton-fibre. Osmic vapour and carmine. Zeiss apochr. oil and oc. 4.

Fig. 15. From chamber eight days in subcutaneous tissue. Plasma-cells adhering to a hair, which had accidentally been allowed to get into the wound. Zeiss obj. D, oc. 2. Osmic acid solution and haematoxylin.

Fig. 16. Inflammatory membrane from chamber eight days in the abdominal cavity; taken from a tenuous portion of the membrane. Four fibroblasts, in a film which is composed of an extremely irregularly arranged network of filaments resembling fine fibrin threads. The processes from the cell-body are continuous apparently with the fibrils of the matrix. Osmic acid vapour and haematoxylin. Zeiss apochr. oil imm. and oc. 4. Outlined with camera lucida.

Fig. 17. Stellate fibroblasts and two leucocytes from same preparation as Fig. 9, more highly magnified. Zeiss apochr. oil and oc. 4. Outlined with camera lucida.

Fig. 18. The modified Ziegler chamber; the sketch shows the actual size employed.

Fig. 19. Portion of the chamber seen edgewise, showing the opening between the cover-glasses. Enlarged 12 times.

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Fig.1

Fig.2.

Fig.3.

Fig.4.

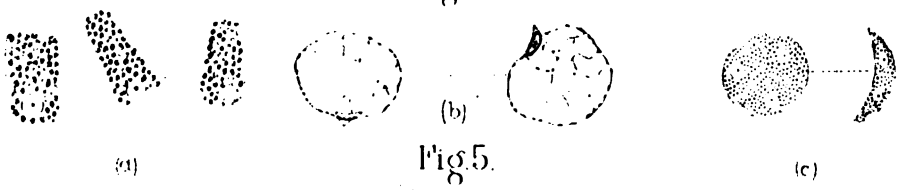
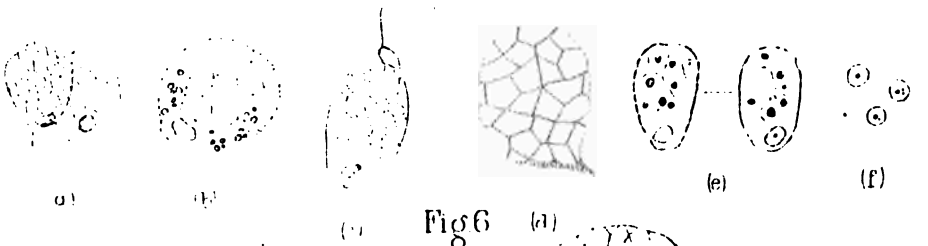


Fig.5.



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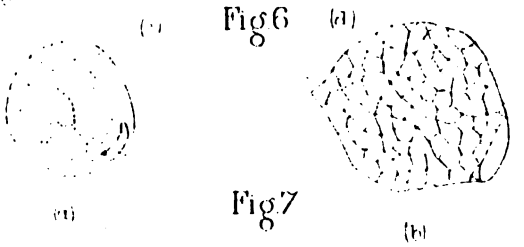

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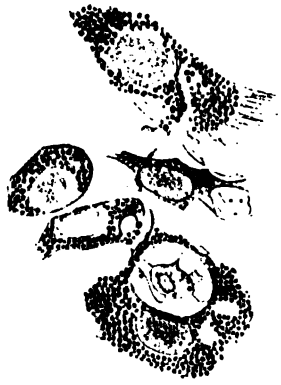

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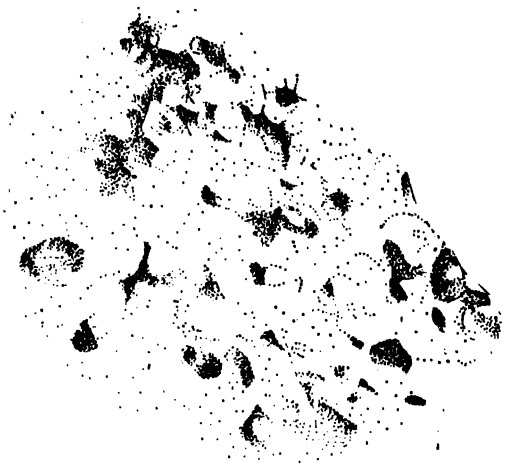

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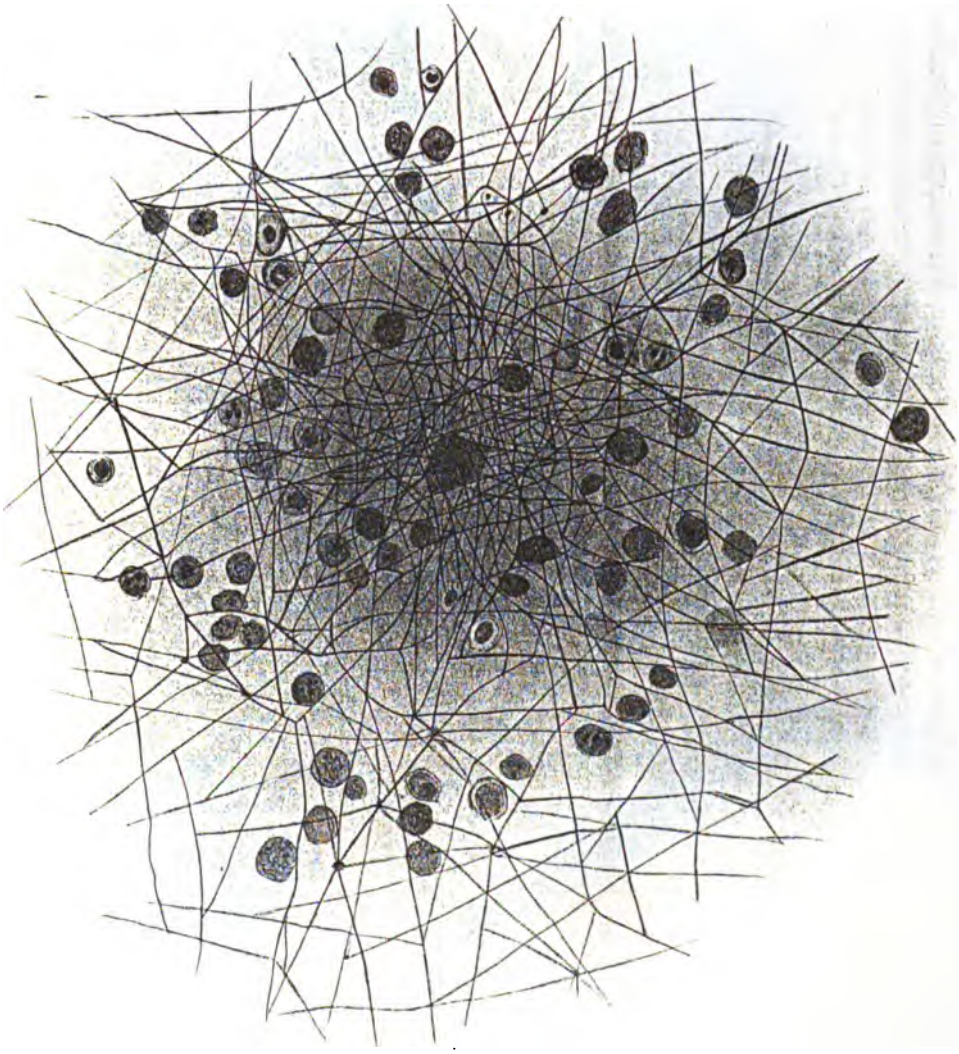

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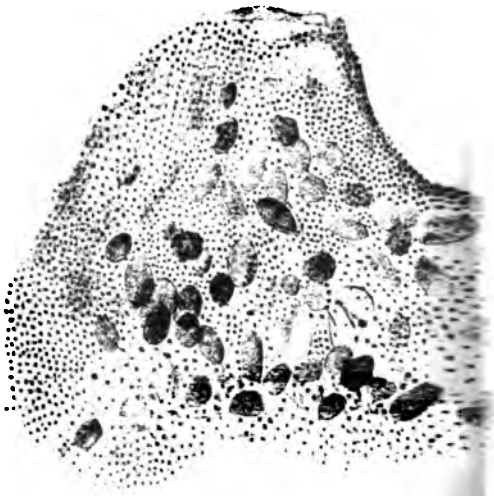

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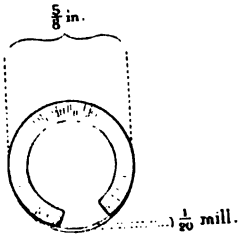

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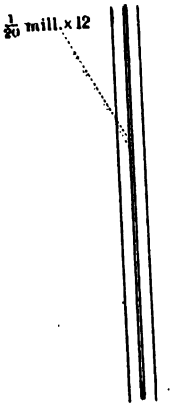

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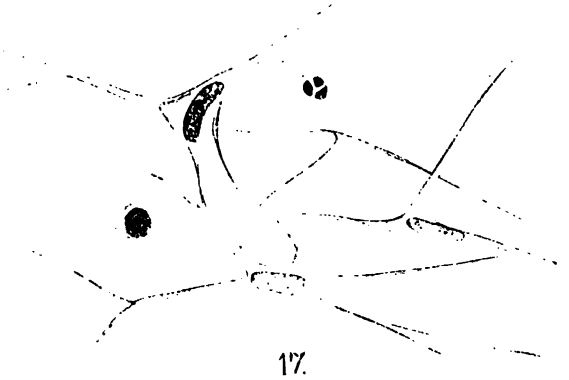

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

1889. No. I.

UNIVERSITY COLLEGE. *Feb.* 9.

1. "On the Innervation of the Pulmonary Vessels," by Drs J. ROSE BRADFORD and H. PERCY DEAN.

Hitherto, although most physiologists have considered that the pulmonary vessels probably possessed a system of vaso-motor nerves, yet no direct experimental proof of the existence of such a system has been obtained. Still less has any evidence been adduced to demonstrate the actual paths by which such nerves, if they exist, reach the lungs. Hence it seemed that the whole question was one deserving a further attempt for its solution.

It was felt by us that the only really reliable method would be to excite one by one the roots of the spinal nerves and to observe the effects of such stimulation on the aortic and pulmonary blood-pressures simultaneously.

The method of the research was demonstrated to the meeting, and is shortly as follows. A cannula placed in the carotid artery in the usual manner was connected with a mercurial manometer. In a similar manner a second mercurial manometer was then connected with the branch of the left division of the pulmonary artery distributed to the lower lobe of the left lung. This vessel was reached from the back by resecting portions of two or sometimes three ribs. In this way a record of the pressure in the left division of the main artery was obtained and also a means of detecting changes of pressure in the main artery. At the same time a minimum amount of lung tissue was thrown out of gear. The upper dorsal nerves were then exposed inside the spinal canal and were ligatured outside the *dura mater*. By cutting through the nerves between the spinal cord and the ligature the peripheral ends could be easily arranged for excitation. The nerves were excited on the right side, the same side as the uninjured lung.

The two blood-pressure curves were recorded simultaneously on the

same blackened surface together with a time-tracing and a lever marking the duration of the excitation.

The animals, in all our experiments being dogs, were anaesthetised with chloroform and morphia, and after the nerves had been prepared a small dose of curare was injected and artificial respiration maintained before opening the chest to insert the pulmonary cannula.

The pressure in the branch of the pulmonary artery is found to vary between 16 and 20 mm. Hg. in different animals. In the main artery it is a few millimetres higher than this.

The pulmonary pressure is very constant in its height, not only in the same animal during the course of an experiment but also in different animals. In this point it contrasts strongly with the aortic pressure, since the latter is very variable in amount after the necessary operative procedure described above. The aortic pressure must fall very low indeed for the pulmonary pressure to be appreciably diminished in amount.

It is evident that our attention must first be directed to the effect produced in the pulmonary blood-pressure by a sudden increase in the aortic pressure. In fact we must see what is the passive effect of an aortic rise of pressure on that of the pulmonary artery. We shall then proceed to consider the effect of reflex stimulation on the pulmonary blood-pressure, and after that to see if there is any mechanism in the central nervous system influencing the pulmonary circulation, and lastly to find out the paths by which the influences emanating from the central mechanism leave the spinal cord. We will therefore consider our subject under the following headings.

A. Passive Effects (purely mechanical).

(1) *The excitation of the peripheral end of a divided splanchnic.*

The rise of systemic blood-pressure is of course considerable, in many cases it is doubled. The rise of pressure in pulmonary artery is not however very marked.

In one experiment the aortic pressure rose from 50 mm.—104 mm. Hg. and the pulmonary rose from 13 mm.—15·5 mm. Hg.

Thus the ratio of aortic to pulmonary rise was about 20 : 1.

(2) *Excitation of peripheral end of the spinal cord divided at level of 7th dorsal nerve.*

In this case the pulmonary rise was always very small compared with the aortic, average ratio 1 : 20.

(3) *Compression of thoracic aorta.*

If compression is only for a short time, 10″, then, although the aortic pressure is more than doubled, yet the pulmonary pressure is not affected at all.

If the compression is longer, 30″, then the pulmonary pressure does rise slightly. As in previous two cases ratio of aortic to pulmonary rise is 20 : 1.

B. Reflex Effects.

(1) *Excitation of central end of a divided sciatic nerve.*

In this case the pulmonary rise varied from $\frac{1}{16}$th—$\frac{1}{18}$th of the aortic rise.

(2) *Excitation of central end of a divided vagus.*

Here the ratio of aortic rise to pulmonary rise was 8 : 1. Thus the pulmonary rise was more than double the rise obtained in the preceding series of passive effects.

(3) Excitation of posterior surface of spinal cord and of posterior nerve rests gave a similar ratio of 8 or 10 to 1.

C. Central Effects.

If the upper part of the medulla be excited there is a very considerable rise in both the aortic and pulmonary blood-pressures.

If now the spinal cord be divided about level of 7th dorsal nerve and the peripheral end be stimulated we get an enormous rise in the aortic pressure as great or greater than by stimulating the medulla, and yet the rise in pulmonary pressure is either absent or very slight.

Again, if the upper part of medulla be now stimulated the aortic rise, as we should expect, is much less than before, about $\frac{1}{3}$, but the pulmonary rise is just as great.

These experiments evidently point to some mechanism in the upper part of medulla oblongata stimulation of which causes a contraction of the pulmonary vessels.

D. Peripheral Effects.

By stimulating the roots of the dorsal nerves from the 2nd to the 7th we get a greater proportionate rise of pulmonary pressure than in any of the preceding experiments. This rise increases as we pass upwards from the 7th to the 2nd nerve root.

Thus the 5th nerve gave an aortic rise of 20 mm. Hg. and a pulmonary rise of 5 mm. Hg.

And the 4th nerve gave an aortic rise of 10 mm. Hg. and a pulmonary rise of 4 mm. Hg.

With the 2nd and 3rd dorsal nerves we often get an actual fall in the aortic pressure with a marked rise in the pulmonary pressure.

E. Effects of Asphyxia.

The aortic and pulmonary blood-pressures begin to rise at the same time, but the latter rises much more gradually than the former. The pulmonary pressure continues to rise for some time after the aortic pressure has begun to fall, and may attain twice its former height.

In conclusion these experiments seem to shew that there is a mechanism in the medulla oblongata, stimulation of which causes a contraction of the pulmonary vessels, and that in the dorsal nerves from the 2nd to the 7th there are vaso-constrictor fibres passing to the pulmonary vessels.

We hope that, shortly, we shall be able to demonstrate the existence or non-existence of vaso-dilator nerves in relation with the pulmonary vessels.

2. "On Transverse Contraction in Muscle." Dr Lauder Brunton observed that there were several phenomena connected with muscular contraction which were not easily explained on the ordinary supposition that muscular fibre, voluntary and involuntary, contracted only in a longitudinal direction, but might be explained on the hypothesis that relaxation was not a mere passive action but was due to contraction in the transverse direction. These phenomena are (*a*) the local dilation which occurs in veins on electrical stimulation. (*b*) Weber's observation of the elongation of a loaded muscle on stimulation. (*c*) The active dilatation of the iris on the application of atropine in animals where no dilator muscle has been satisfactorily shewn to exist. (*d*) The fact observed by Dr Cash and himself that theine may cause in muscles either enormous contraction, no contraction at all, or actual relaxation, a phenomenon which seemed to indicate the presence in muscle of two opposing forces which may either counterbalance one another or cause positive elongation or contraction. (*e*) The observation of von Kries[1] that when a muscle has been made to contract by stimulation, a second stimulus affecting the muscle during its relaxation does not at once arrest the descent of the curve, but only does so when the second stimulus by itself would cause a marked action.

3. Prof. V. Horsley and Dr Beevor made a communication on the Localisation of Areas in the Cerebral Convolutions of the Orang.

[1] *Archiv für Anatomie und Physiologie.* Parts V. and VI. p. 547, 1888.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

1889. No. II.

CAMBRIDGE. *March* 9.

J. N. LANGLEY, F.R.S., "On the Preservation of Mucous Granules in Secretory Cells." On irrigating fragments of fresh mucous gland with osmic acid I was struck with the fact that most of the granules preserved their form—although swelling up—when only a small amount of osmic acid solution was added, but that the granules disappeared on adding a large amount of osmic acid. This led me to try the effect of placing over an osmic acid solution a freshly teased out specimen of a mucous gland, the gland being teased in a 2 to 5 p.c. sodium chloride solution, in which the granules remain for some time unaltered. In this way I obtained permanent preparations showing a certain number of mucous spheres (the granules) both isolated and in the cells. This induced me to try whether the mucous gland could be preserved for the purpose of preparing microscopic sections, by hardening it in osmic acid vapour. The use of osmic acid vapour to fix and harden tissues is not new, Ranvier in especial has recommended it in particular cases. But its use in the case of certain mucous glands gives very striking results. In the first place the mucous granules can be preserved in a more or less spherical form, and in the second the mucous granules are then stained with most reagents far more deeply than any other part of the tissue. The success of the preparation depends a good deal upon the details of the method; it depends also, apparently, in part upon the gland, for I have obtained more constant results with the orbital gland of the dog, than with other mucous glands. The method which has so far given me

the most satisfactory results is as follows:—A fine needle with a silk thread is passed through a small lobule of the fresh gland of an animal which has been killed by bleeding whilst under the influence of an anæsthetic, or which has been killed by decapitation; the lobule is cut out, lowered by the silk thread passing through it into a bottle half full of 2 p.c. osmic acid. When the lobule is a short distance from the surface of the fluid, the stopper is inserted, which, as it clamps the thread, keeps the piece of gland from touching the fluid. In a day, the tissue, now hardened throughout is washed for a few minutes with water, placed in 30 p.c. alcohol and in 50 p.c. alcohol for about a quarter of an hour, in 75 p.c. alcohol and in 95 p.c. alcohol for about half an hour; in absolute alcohol for one to two hours, in benzol for half an hour to an hour, and then imbedded in hard paraffin. Serial sections are cut, and then either (*a*) fixed on a glass slide with egg albumin, stained with methylene-blue and mounted in Canada balsam, or (*b*) treated with benzol or turpentine to dissolve the paraffin, passed through alcohols of various strengths to water and stained with a strong aqueous solution of methylene-blue, and finally—after the usual treatment—mounted in Canada balsam.

In all other methods of hardening mucous glands which I have tried, the mucous granules are more or less altered, being either swollen up to form a clear mass, or being shrunken to irregular small clumps. By the method given above the mucous granules in the stained specimen are very obvious, and further in a stimulated gland many of the alveoli show an inner zone of granules stained deep blue and an outer zone stained of a brownish tint. Even in the unsuccessful preparations, in which the granules are not separately distinguishable, the mucous portion of the cell is unusually well marked in stained specimens.

In the mucous cells of the mucous membrane of various lower vertebrates in which granules can be made out in the fresh state, the granules can often be preserved by the vapour of osmic acid. For certain special points in other glands, particularly in the parotid, the method is useful, but this together with further details with regard to its employment on mucous cells I propose to deal with after making further observations.

Communications were also made by Dr SHERRINGTON, Prof. ROY and Mr ADAMI, and Dr HUNTER.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

1889. No. III.

KING'S COLLEGE. *May* 11.

1. M. ANATOLE PILTAN demonstrated his method of recording the movements of vocalisation. He had made experiments on various singers by means of an elastic bag or special tambour held in contact with the walls of the chest and of the abdomen and with the front of the larynx by means of a belt, or by a bag adapted to the corset. The bags communicated the slightest movement of the chest or abdomen to a tambour writing on the blackened surface of a revolving cylinder, where they were recorded in the form of curves.

It was observed that voice depends on effort, and that the effort of a good singer consists in upheaving the chest and avoiding contraction of the muscles of the abdominal walls, and that the diaphragm (being lowered according to the pitch or the intensity of the sound) thus becomes the regulator of the expiratory movement.

The longest duration in this produced effort insures the best voice.

The degree of regularity with which these movements are performed determines the evenness of the voice; and the effort of good singers consists less in uttering sounds more or less intense, as in crying, for instance, than in holding them steadily for the longest possible duration. On the contrary, most of the singers having a bad voice called into action the muscles of their abdominal walls.

Efforts are of three kinds:

(1) The general or thoraco-abdominal effort. This first variety includes those movements in which muscles have to interfere for pushing, lifting, pulling, carrying, &c. or again those producing a series of bad tones.

(2) The abdominal or expulsive effort.

(3) The thoracic effort or singer's effort.

The curves registered in simple breathing, speaking low and high, in coughing, groaning, crying, in a violent effort, in blowing in musical instruments, in singing with a bad voice, were demonstrated; and the latter when compared with those of singers having a really *good voice* showed marked differences.

Besides, the author has observed with the aid of a spirometer and a chronograph that a bad voice could not sustain a sound loudly more than 5 or 6 seconds, whilst a very good voice could sustain it for 30 or 35 seconds.

2. C. J. MARTIN demonstrated the method of recording, and exhibited graphic tracings of, the rate of secretion of the bile in the case published in Mr Copeman's paper on Bile in the *Journal of Physiology*. The tracings were confirmatory of Mr Copeman's results.

3. Communications were also made by Mr SHORE on Taste and Mr FRANCE on brain and cord degenerations after lesion of cortical motor areas.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

1889. No. IV.

OXFORD. *July* 6.

DR WALLER (for Miss Brinck) communicated the results of experiments made by her on the nutrition of excised skeletal muscle of the frog. The object of these experiments was to test on skeletal muscle the nutritive action of various proteid fluids, in continuation of the results which Kronecker and his pupils (Martius, v. Ott, N. Popoff, J. Brinck) have obtained on cardiac muscle. The main result of Miss Brinck's experiments is to extend to skeletal muscle the conclusion which Kronecker has established on cardiac muscle, to the effect that serum-albumin is the nutritive proteid *par excellence.*

Egg-albumin and peptone solutions have no restorative action on muscle, contrasting with serum-albumin or blood solutions which are capable of restoring muscular contractility from zero to its normal amount; the restoration may be effected more than once, as is shewn by the following experiment:

EXP. *March* 15, 1889. Frog. Sartorii suspended in two vessels containing respectively 50 c.c. egg-albumin solution about 1 %, 50 c.c. serum-albumin solution about 1 %.

Time.	Height of contraction of muscle A.		Height of contraction of muscle B.	
0	.....20 mm.	(in serum solution)	.....20 mm.	(in egg solution)
2 hours	.....21 „		..... 3 „	
2 „	.....	(solutions reversed)		
3 „	.....18 „	egg	..... 4 „	serum
4 „	.....12 „	egg	.....12 „	serum
4½ „	..... 4 „	egg	.....14 „	serum
5½ „	..... 2 „	egg	.....15 „	serum
5½ „	.....	(solutions reversed)		
5½ „	..... 3 „	serum	.....17 „	egg
7 „	.....14 „	serum	..... 14 „	egg
9 „	.....17 „	serum	..... 2 „	egg
9 „	.....	(solutions reversed)		
18½ „	..... 0 „	egg	.....20 „	serum
19 „	.....	(solutions reversed)		
19½ „	..... 7 „	serum	.....22 „	egg
27 „	.....20 „	serum	..... 0 „	egg
27 „	.....	(solutions reversed)		
29 „	... ..17 „	egg	.....18 „	serum
29½ „	.....13 „	egg	.....18 „	serum
32 „	..... 2 „	egg	.....18 „	serum
32 „	.....	(solutions reversed)		
41 „	.....14 „	serum	..... 0 „	egg
45 „	.....14 „	serum	..... 0 „	egg
45 „	.....	(solutions reversed)		
50 „	..... 0 „	egg	.....15 „	serum
53 „	..... 0 „	egg	.....16 „	serum
53 „	.....	(solutions reversed)		
63 „	..... 6 „	serum	..... 0 „	egg
68 „	..... 6 „	serum	..... 0 „	egg

Thus in the course of 68 hours the solutions were changed 8 times, in each case with the result—decline of contractility in the egg-albumin solution, recovery of contractility in the serum-albumin solution.

Other communications were also made.

54-31-25

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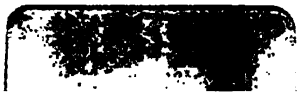
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